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On the Distribution of Electric Potential on the Seedling of *Vigna sesquipedalis* and its Change by the Light Stimulation

By Hisashi OKAMOTO*

岡本 尚: ミトリササゲ幼植物の生体電位分布とその光刺激による変化について

Received June 18, 1954.

It is strongly desired to obtain informations about the distribution of substances and its shift with metabolic activity in a plant body as a whole and to pursue them within a short time in reference to the extensive studies on the metabolism in germination in this laboratory (1), (2). For this purpose I attempted to investigate the distribution of electric potential on *Vigna* embryo during its germination-stage, and its correlation with the growth.

It was also found in these works that the resting potential undergoes a sudden change by a contact stimulation or by a light irradiation. Some characteristic properties of this excitation caused by light are described.

In order to comprehend the meanings of the potential distribution and its changes in relation to the metabolic activity of tissues and their growth rates, it is also indispensable to obtain informations about histological structures of the plant, so that we may become acquainted with the qualitative spatial differentiation of functions of tissues on the one hand and the chronological as well as topographical variation in the state of growth on the other hand.

Methods

Material:—Seeds of *Vigna sesquipedalis* (1) were immersed for an hour in 0.1% solution of Uspulun, soaked for 5 hours in tap water, then sown on washed sand in porcelain pots and incubated in the dark at 30°C. After a while the seeds sprouted and etiolated plants grew with remarkable elongation velocity until about 6 days after seeding, though differentiation of shoot apex took place only to a small extent. Some plants were cultured for comparison under the scattering light in the room. Measurements were carried out with plants cultured in the dark as well as those in the light in various stages of germination during 5 days.

Apparatus:—An electron tube potentiometer of a special design (3), (4) was employed, which was made up of a bridge part comprising an electron tube for the potentiometrical use (UX-54 or 54B, TOSHIBA Elec. Co.) and a potentiometer of high precision (YOKOKAWA Elec. Co., Model P-1) combined with an highly sensitive galvanometer (YOKOKAWA Elec. Co., Model D-3A).

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All the circuit is illustrated in Fig. 1.

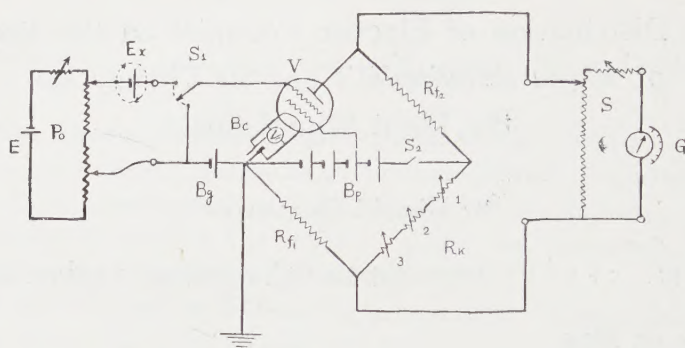


Fig. 1. Circuit used for the measurement

Po: potentiometer, S: universal shunt, G: galvanometer, V: UX-54, E: battery, 2 volt 12AH, Ex: EMF to be measured, Bg: battery, 1.5 volt, Bc: battery, 2 volt 12AH, Bp: battery 1.5 volt $\times 4$, Rf1: fixed resistance 10K Ω , Rf2: fixed resistance 50K Ω , Rk: variable resistance 25 Ω , 100 Ω , 2000 Ω , S1: mercury change over switch, S2: ordinary switch, V: voltmeter.

Although the potentiometer might be operated owing to the compensation principle, its circuit components were changed only in the calibration and in a series of measurements kept unchanged in most cases; thus the potential difference was read directly from the displacement of the galver index after calibration.

As main sources of error in the measurement polarization and high ohmic resistance of the object (about 100K Ω per cm of organ length) are to be accounted. The former can be avoided if the measurement is carried out without appreciable flowing of current through the object, realizing this perfectly by putting the inquired E.M.F. (Ex) into the grid circuit of the bulb and examining the variation of its plate current. To avoid the latter effect its grid resistance (Rg) must be high enough to surpass that of the object (R). This technical error can be estimated by the formula:

$$\Delta E = Ex \frac{R}{R_g + R}$$

Then, if Rg be 100 times greater than R, the error ΔE will be about 1% of the true value. For this purpose UX-54 with high grid resistance of 10¹⁴ Ω was fairly available. If its grid circuit is strictly insulated, ΔE caused by high resistance of the object becomes negligibly small, even in such a extreme case as 1M Ω of it.

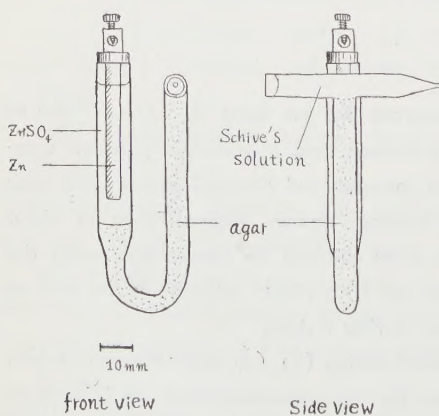


Fig. 2. Non-polarizing electrode, Zn/ZnSO₄/agar/Schive's solution.

about 50-60 $\times 10^3 \Omega$.

As the measuring electrodes non-polarizing electrodes of Zn/ZnSO₄ were employed (Fig. 2). The characteristic properties of them were satisfactory stability, very small mutual potential difference (at most 1-2 mV) and their resistance of

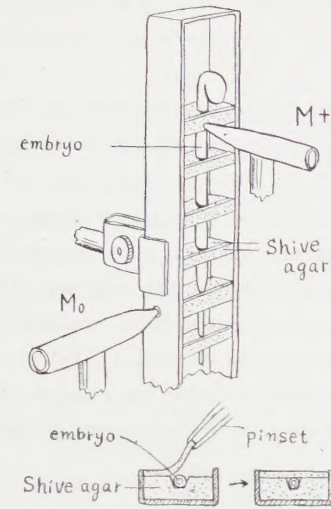


Fig. 3. above; Arrangement of the electrodes by the indirect method.

Mo; fixed electrode, M+; tracing electrode.

below; Embedding of the plant in the agar keel-blocks.

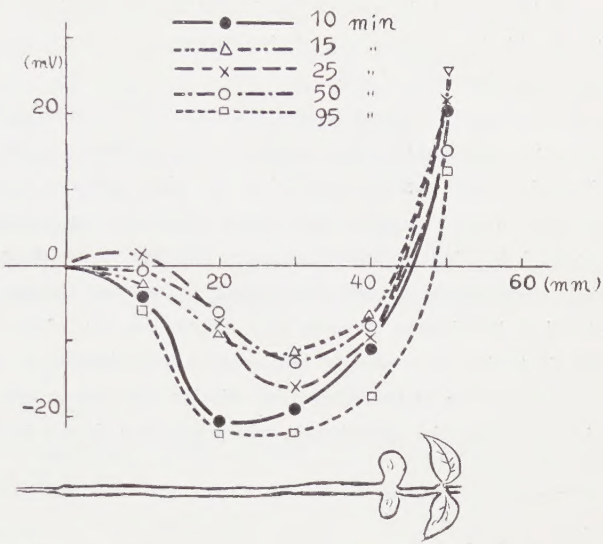


Fig. 3''. Fluctuation of the potential distribution within 95 minutes. Each curve represents a distribution of potential along the growth axis, being referred to the electrode on the boundary of hypocotyl and radicle.

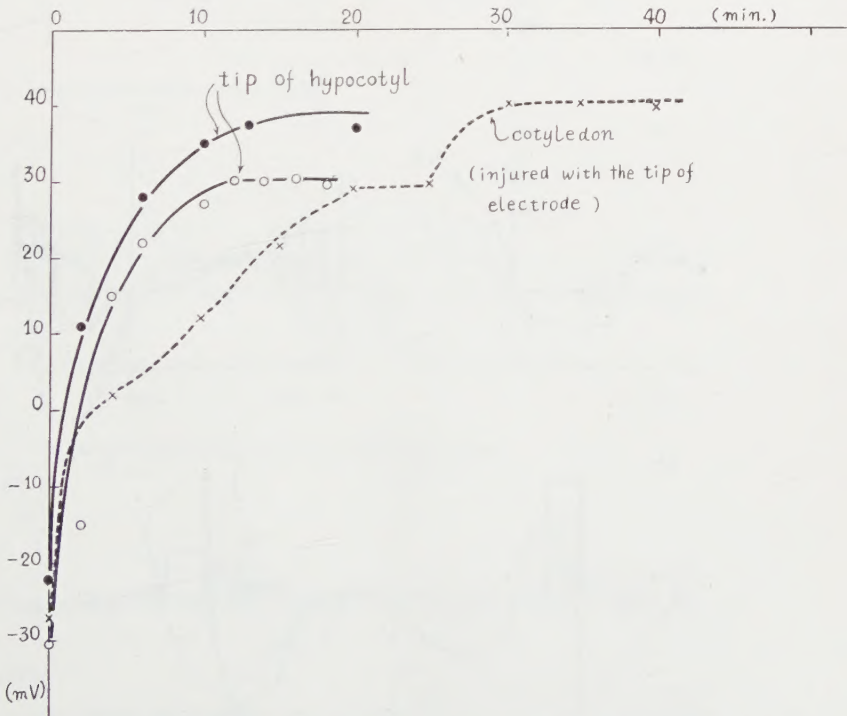


Fig. 3'. Fall of the resting potential by a contact of the electrode at the time 0 and process of its recovery.

Reliability of the measurements can be examined by connecting the inquired E. M. F. inversely. ΔE should then be one half of the difference of both values given by this procedure. This error has never been over 1 mV.

Experimental Arrangements:—Another factor which might disturb the measurement is the excitation arisen in the tissue caused by the mechanical stimulation on the contact of the measuring electrodes. The resting potential of the plant exhibits a sudden fall by such a disturbance and recovery of it proceeds very slowly (Fig. 3'). In hitherto published reports on the problem of potential distribution there have been somewhat confusions in this respect (5). In order to avoid such a disturbance, a method of indirect contact was adopted in this study; that is, the material was placed in keel-blocks of many agar pieces (Fig. 3). After the recovery from the initial excitation, the electrodes were brought into contact with these agar pieces. One of the electrodes (Mo) was

Distribution of the elongating velocity was determined by the marks of Indian ink at intervals of 5 mm along the growth axis, which had been drawn immediately after the measurements of the

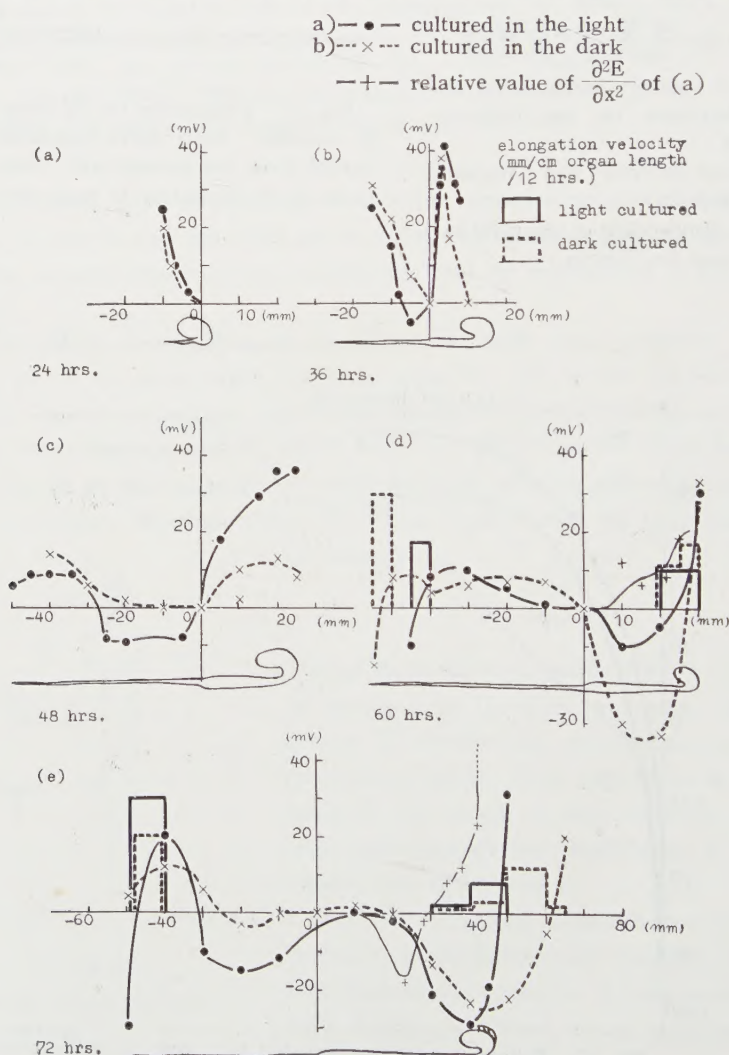


Fig. 4. Distribution of the potential

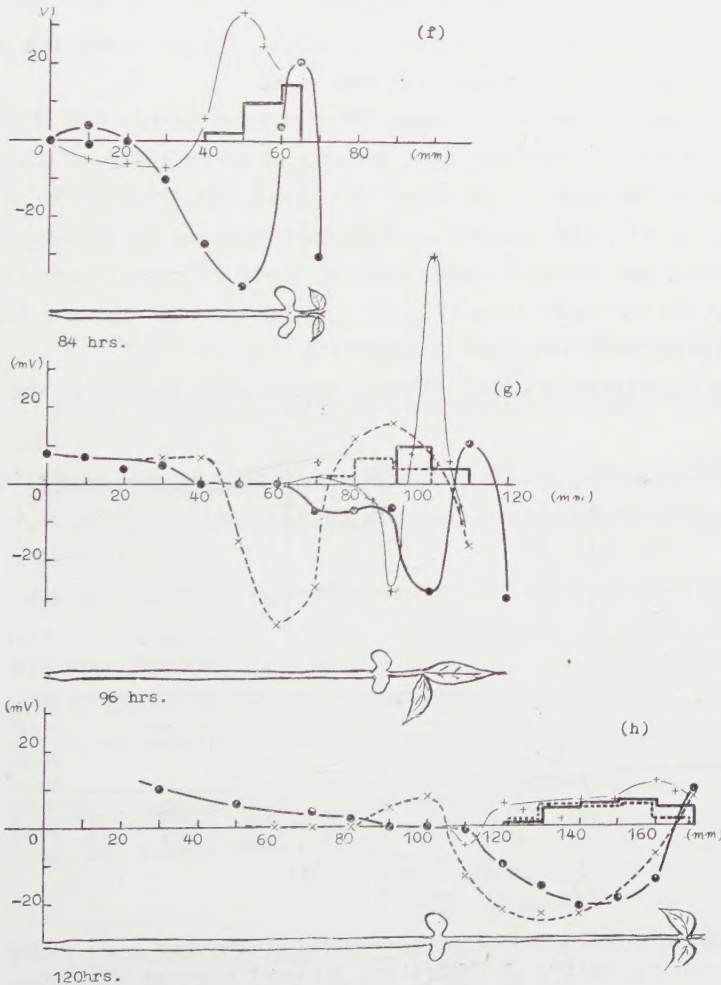
always fixed on the boundary of hypocotyl and radicle, another one (M+) was transferred from one agar piece to another along the elongation axis successively, thus the resting potential differences between Mo and M+ were measured. The potential distribution thus measured was satisfactory stable and of an uniform type (Fig. 3''). All measurements were carried out in a moist glass chamber furnished with openings available for the outlet of the leads and measuring operations, keeping the material vertically in the air.

potential differences. Morphological observations under the microscope were carried out mostly on stained preparations. Navashin's solution was used for the fixation and sections obtained through ordinary paraffin method were stained with hematoxyline combined with iron-alum.

Results

1. Distribution of the Resting Potential.

The distribution of the electric potential, which varied with culture age during 5 days after germination, are illustrated in Fig. 4 a-h. It is suggested from these



varying with the growth stage.

data that the differences in the culture conditions (whether in the light or dark) have no serious influence upon the type of the potential distribution. Apparent disparities in the forms of these two curves (for instance, what is seen in the culture stage of 96 hours), however, are to be ascribed to the difference in the state of growth owing to the dissimilar conditions of the plants.

The form of the potential distribution on seedlings in earlier stage (36 hours) coincides nearly with what is shown on those of *Lupinus albus* by Ramshorn (6). The potential distribution changes, however, its form conspicuously after 60 hours, especially on the hypocotyl part. A valley of the potential curve appears at the elongating part of the hypocotyl, and whose relative position migrates upwards with its progressive growth and at last it shifts completely upon the growing part of the epicotyl, when the elongation of the hypocotyl has utterly ceased.

Concerning the radicle, the gradient of the potential is too steep in the elongating zone when determine its exact form by the aforementioned method.

2. Influence of Light upon the Resting Potential

Besides mechanical stimulation, a disturbing effect of light upon the resting potential had been recognized in the course of this work.

Red light of a lamp, whose wave length is restricted above 6300 \AA and its intensity is 5 C.P., however, possesses, at a distance of about 10 cm no effect upon the resting potential of the plant in any stage of culture. On the other hand, white light from a bulb of 60 watts caused a remarkable fall of the potential of the hypocotyl tip, although the standard point (junction of the hypocotyl and radicle) is similarly irradiated at the same time (Fig. 5). In this case the distance from the light source was appropriately enlarged to about 1 m.

It is interesting to be perceived that the more vigorous the growth is, the higher

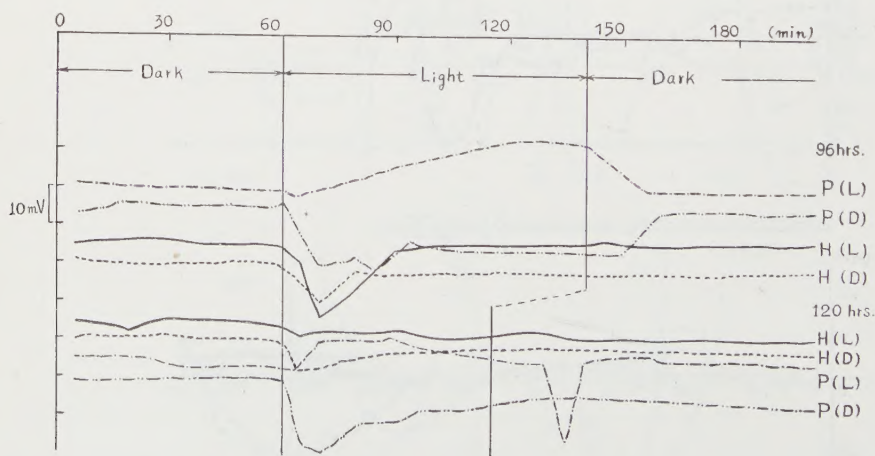


Fig. 5. Influence of light upon the resting potential at a certain point on the hypocotyl(H) and plumule(P). L(Light) and D(dark) represent the culture condition respectively.

the reaction intensity, or in another expression the more sensitive the tissue concerned. Behavior of the plumule is somewhat strange; the type of the light sensitivity of the dark cultured one is the same as that of the hypocotyl, while the potential level of the light cultured one disclosed no tendency of a fall by the irradiation, but a steady ascent. So-called "off effect", or potential fall with interruption of lighting is also only seen in this light cultured plumule.

3. Histological Matters.

Through microscopic observations on the tissue structures of the embryonic plants we were able to ascertain clearly that the elongation and the corpulense of the hypocotyl and epicotyl depend only upon the cell growth, and proliferation of the cells by division takes no part in this case. This conclusion would be derived from following experimental facts.

Bean embryos in the culture stages of 48, 72 and 96 hours were employed as materials. The occurrence of elongation is restricted in the zone of 10-20 mm long under the neck of cotyledon. This growing process is shown in Fig. 6 schematically.

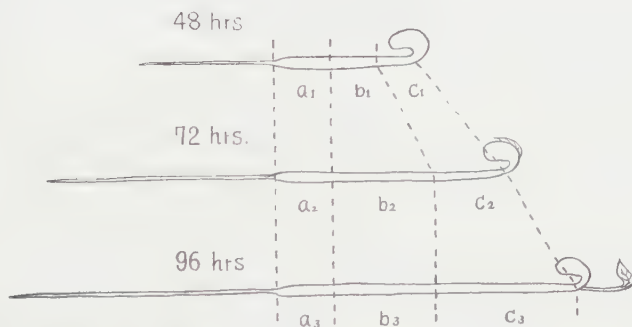


Fig. 6. Schematic illustration of the growing process of the seed embryo.

Though we might have no complete data as yet, following relations were proved to exist substantially (see Table I and II). As regard to the cross section; there exist relations,

$$a) \quad Na_1 = Na_2 = Na_3, \quad Nb_2 = Nb_3 \quad \text{where N's represent number of cells in a cross section;}$$

$$b) \quad Nb_1 = Nb_2, \quad Nc_1 = Nc_2, \quad Nc_2 = Nc_3$$

As regard to the length section, there exist relations,

$$a) \quad la_1 = la_2 = la_3, \quad lb_2 = lb_3 \quad \text{where l's represent average length of cells in a length section,}$$

$$\text{and } b) \quad \frac{lb_1}{lb_2} = \frac{Lb_1}{Lb_2}, \quad \frac{lc_1}{lc_2} = \frac{Lc_1}{Lc_2}, \quad \frac{lc_2}{lc_3} = \frac{Lc_2}{Lc_3}$$

where L's represent the length of the regions of the plant body.

Values of L_1/L_2 and L_2/L_3 ought to disclose inevitably somewhat appreciable fluctuation when they are compared with the ratios of cell growth themselves in a certain

range of the corresponding part, because distribution of the elongation velocity is naturally not homogeneous in any finite range. Values of these ratios shown in the Table II mean their maximum and minimum value, each corresponding to the case where length of slowly elongating part of the responsible region is neglected from the denominator or not.

Table I. Average number of cells on a diameter, A or B. ($A \perp B$), in a cross section

	Nb ₁	Nb ₂	Nb ₃	Nc ₁	Nc ₂
A	34	29	32	28	32
B	30	30	28	27	23

Table II. Average length of cells and elongation ratio, in a length section

Average length of cells (μ)				
	Epidermis	Cortex	Stele	Conducting tissue
lb ₁	14.4	21.6	25.2	108.0
lb ₂	67.6	87.6	—	360.0
lb ₂ /lb ₁	4.68	4.05	—	3.5
Lb ₂ /Lb ₁		2.5	4.0	
lc ₂	16.4	26.4	27.2	128.0
lc ₃	88.8	110.0	126.0	656.0
lc ₃ /lc ₂	5.41	4.17	4.63	5.08
Lc ₃ /Lc ₂		3	5	

Synthesis of cytoplasm seemed not to take place in the hypocotyl part of etiolated plants at least 48 hours after the seeding so far morphological observations are concerned. Thus it may be admitted that the essential work performed in their growth is the so-called turgor work, or the expansion of cells by the osmotic pressure of the solutes in the cell sap besides synthesis of cell-wall.

It is also worthwhile to be mentioned in this place that the microscopic examinations of the embryonic tissues revealed the presence of strongly basophilic granules in cotyledon, which are quite distinguishable from starch granules and later on disappear with the culture has proceeded (Fig. 7). They seemed not to be anything like lipid, because Sudan III cannot stain them. Dealing with 1N HCl at 60°C for ten minutes no appreciable change could be recognized. The nature of these granules

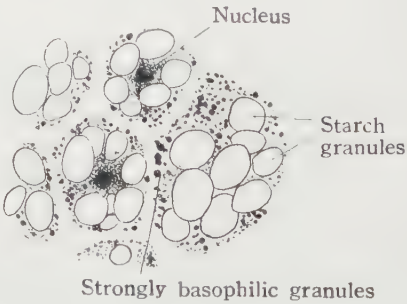


Fig. 7.-(i) Strongly basophilic granules in the cotyledon. $\times 150$

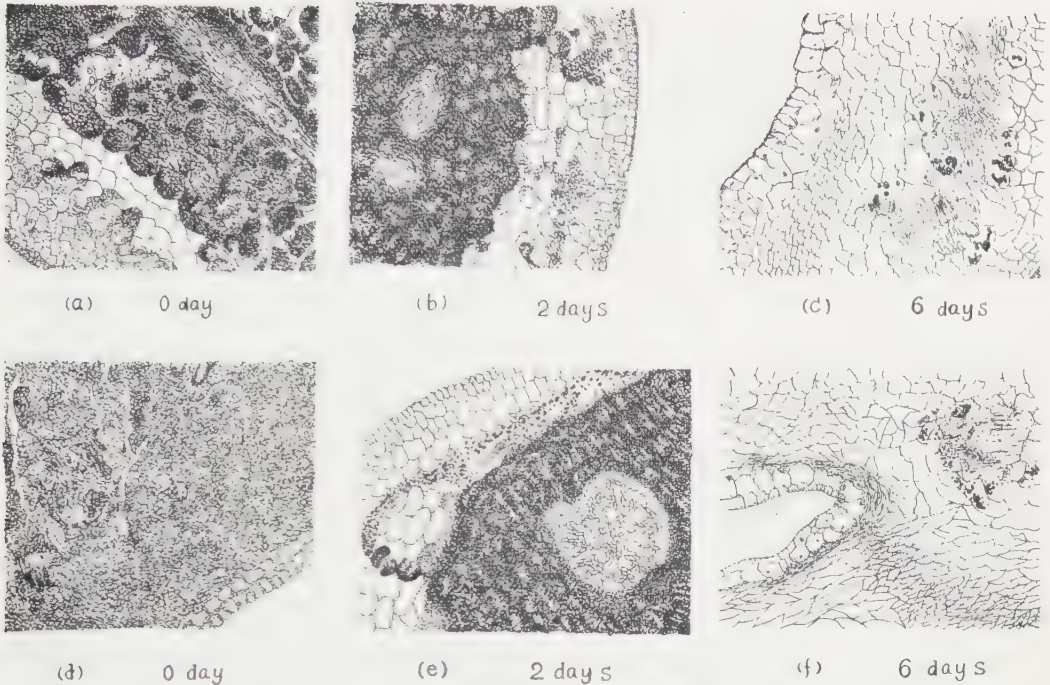


Fig. 7-(ii) Their disappearance with the proceeding of the growth
 (a) (b) (c)—control, (d) (e) (f)—dealt with 1 N HCl at 60°C for ten minutes. $\times 50$

and its physiological meaning are obscure in the present stage of investigations, but we will suppose that such a morphological significance can possibly refer to the known prominently characteristic function of this organ (1), (2), (7), (8).

Discussion

Bünning has stated in his well-known text-book that „Zum Beispiel können wir den Erfahrungssatz benutzen, daß stark atmende Zonen gegen wenig atmende durchweg elektrisch positiv sind.Jedenfalls läßt diese Erfahrungsregel, eine andere Parallellität interessant erscheinen; nämlich die zwischen elektrischer Positivität und Wachstumsintensität, der also eine Übereinstimmung zonaler Atmungs- und Wachstumsunterschiede entsprechen muß.“ (9)

The contents of his description seem to concern with the type of distribution which can be seen in earlier stage (culture of 24–48 hours) in my case. On the other hand, in later stage of germination (about 60 hours) the distribution of the elongation velocity discloses any zonal coincidence neither with the distribution of electric potential, nor with its gradient. So far as hypocotyl and epicotyl are concerned, it coincides best with the divergence of the potential gradient, that is, elongation is observed only in the region where the second derivative of the electric

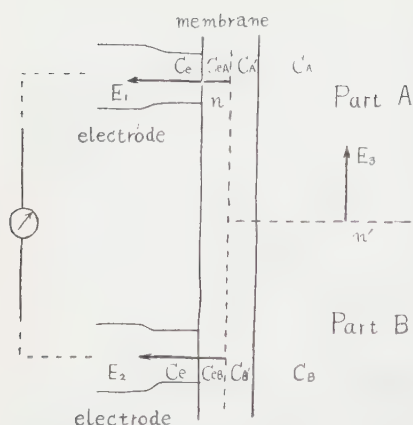


Fig. 8. Schematic illustration of the mechanism through which the electric potential difference $E_1 - E_2 + E_3$ between two parts (A and B) in a living inhomogeneous system can be generated.

potential concerning the elongation axis has a positive value. This experimental fact can be explained reasonably from the following considerations.

The source of the electric potential difference induced by the method of surface induction cannot be regarded but as the unequal spatial distribution of ions, so far as our present physicochemical knowledges indicate. Then electric potential difference (E) between any two parts of a biological system A and B must be represented as follows, assuming a boundary region of a nature of double layer. As illustrated in Fig. 8, the potential difference is considered to be separated into three parts E_1 , E_2 and E_3 :

$$E = E_1 - E_2 + E_3$$

The former two in the right side of this equation represent falls of the phase boundary potential, which may be given by following representations (see Fig. 8):

$$E_1 = \frac{RT}{F} \sum_i \left(\frac{1}{Z_i} \ln \frac{C_{Ai}}{C_{ei}} + \frac{1-n_i}{Z_i} \ln \frac{C_{eAi}}{C_{Ai}'} \right)$$

$$E_2 = \frac{RT}{F} \sum_i \left(\frac{1}{Z_i} \ln \frac{C_{Bi}}{C_{ei}} + \frac{1-n_i}{Z_i} \ln \frac{C_{eBi}}{C_{Bi}'} \right)$$

E_3 is the potential difference referred to the internal concentration chain of ions and can be written in the formula:

$$E_3 = \frac{RT}{F} \sum_i \frac{n_i'}{Z_i} \ln \frac{C_{Bi}}{C_{Ai}}$$

In these formulae C_i represents concentration of the i -th kind of ion, Z_i represents its ionic valence (positive for cation and negative for anion) and n_i and n_i' the transport numbers of them, concerning outer surface and inside of the system respectively. From these equations we shall have

$$E = \frac{RT}{F} \sum_i \left(\frac{1}{Z_i} \ln \frac{C_{Ai}}{C_{Bi}} + \frac{1-n_i}{Z_i} \ln \frac{C_{eAi}}{C_{eBi}} \frac{C_{Bi}'}{C_{Ai}'} \right) + \frac{RT}{F} \sum_i \frac{n_i'}{Z_i} \ln \frac{C_{Bi}}{C_{Ai}}$$

If we can assume approximately two conditions:

$$\frac{C_A}{C_B} = \frac{C_A'}{C_B'} \quad \text{and} \quad C_{eA} = C_{eB} = C_e$$

to hold, the above formula will be reduced to a more simple one, that is

$$E = \frac{RT}{F} \sum_i \frac{n_i - n'_i}{Z_i} \ln \frac{C_{Ai}}{C_{Bi}} = \frac{RT}{F} \ln \prod_i \left(\frac{C_{Ai}}{C_{Bi}} \right)^{\frac{n_i - n'_i}{Z_i}}$$

The argument of the logarithmic function corresponds to the ratio of the total activity of ions for related two points. As we should always consider the potential difference E as related to a fixed standard point, as is the case in the description of experimental results, then we can write the relation in a trivial form

$$E - E_o + \frac{RT}{F} \ln \epsilon \dots \dots \dots (1)$$

where E_o means potential of the standard point and ϵ the total activity of ions at the point of potential difference E .

An existence of such a potential difference in any biological system depends upon the unequal distribution of ionic concentration maintenance of which requires stationary conversion of energy and consumption of free energy. With the intention to elucidate correlations among growth, potential difference and energy conversion, following theoretical treatment was worked out.

In an embryonic plant such as seedling of *Vigna sesquipedalis*, ions and metabolic substrates must be continuously flowing along the elongation axis. It can be assumed that such a flow obeys simple law of diffusion as a whole except in the conducting tissue.

Total activity of ions at a point, whose position on the elongation axis is represented by x , may be expressed by $\epsilon(x)$ in the same meaning as mentioned above and concentration of metabolic substrates by $C(x)$. p will denote energy liberated in the unit time and unit volume at the point. Some parts of p are converted into the work to absorb (from the conducting tissue, if it may exist in the primitive form) and generate ions, denoting this q . Furthermore some parts of q are used in the osmotic work of ions w which participate in the plastic expansion of the cell wall. Then we can set up following equations ;

$$\begin{aligned} \mu_c \frac{dC}{dt} &= \mu_c k_c \frac{\partial^2 C}{\partial x^2} - p - \mu_c s \\ \mu_\epsilon \frac{d\epsilon}{dt} &= \mu_\epsilon \frac{1}{R} \frac{\partial^2 \epsilon}{\partial x^2} + q - w \end{aligned}$$

where t represents time, s means the part of metabolite that is spent as material in synthesizing cell constituents, μ_c and μ_ϵ are the chemical potentials corresponding to C and ϵ respectively, k_c and $\frac{1}{R}$ being the diffusion constants. In the stationary state we must assume

$$\frac{dC}{dt} = 0 \quad \text{and} \quad \frac{d\epsilon}{dt} = 0$$

Setting $p + \mu_c s = p'$, we may write

$$\mu_c k_c \frac{\partial^2 C}{\partial x^2} = p'$$

$$\mu_e \frac{1}{R} \frac{\partial^2 \epsilon}{\partial x^2} + q = w$$

Summing up these two equations, we can write as a general expression

$$\mu_c k_c \frac{\partial^2 C}{\partial x^2} + \mu_e \frac{1}{R} \frac{\partial^2 \epsilon}{\partial x^2} = w + p' - q$$

where $p' - q$ corresponds to the energy other than w , that is the endergonic work for the synthesis of cell substances on one hand and the part of energy lost in the form of heat, or the so-called energy of maintenance on the other hand. These relations

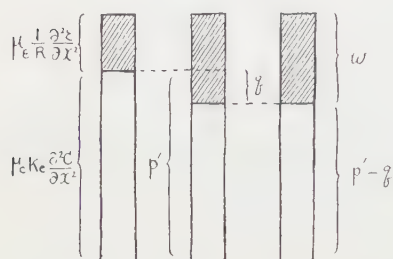


Fig. 9. Schematic illustration of the energy balance among the transport of substances by diffusion, turgor work, catabolic and anabolic conversions.

can be illustrated schematically in Fig. 9. $\frac{1}{R}$

$\frac{\partial^2 \epsilon}{\partial x^2}$ means the balance of departure and entrance of ions in the unit time and unit volume and is approximately equal to the osmotic work of ions at stationary state. It is, therefore, acceptable that any part of an organ where $\frac{\partial^2 \epsilon}{\partial x^2}$ has high positive value theoretically coincides with that of high elongation velocity.

On the other hand, from equation (1), we have

$$\frac{\partial^2 \epsilon}{\partial x^2} = \left\{ \frac{F}{RT} \frac{\partial^2 E}{\partial x^2} + \left(\frac{F}{RT} \right)^2 \left(\frac{\partial E}{\partial x} \right)^2 \right\} \exp \left\{ \frac{F}{RT} (E - E_0) \right\}$$

It can be concluded from this equation that $\frac{\partial^2 \epsilon}{\partial x^2}$ must be positive when $\frac{\partial^2 E}{\partial x^2}$ is positive and it will vary nearly parallel with the latter. Thus from the assumption that the electrical potential distribution is determined by the distribution of ionic concentration, aforementioned experimental results showing coincidence of $\frac{\partial^2 E}{\partial x^2}$ with the distribution of elongation velocity can generally be understood.

The abrupt changes of the distribution of potential by light is a noteworthy fact, especially in the sense that various functional rolls of light in the plant may be apprehended through quantitative analysis on this phenomenon to the certain extent. In general the light stimulation appears to play an important roll in plant life. For instance, so-called "light-growth reaction", or phototropism and photonasty and fall of velocity in the protoplasmic movement by irradiation are regarded as the most general reactions that must occur in every tissues of the plant in principle. Moreover, relations between light and germination have been demonstrated to exist by many authors in various plants. In this respect the light stimulating effect on

the resting potential is supposed to be also a light reaction of the most general nature.

It is desirable to find out what substance is responsible for absorbing light. Although precise investigations on the action-spectrum have not yet been carried out, we see reason to assume that the most probable substance may be a carotenoid in this case because of the ineffectiveness of the red light.

Finally it would be worthwhile to be noticed that the unique properties in the light sensitivity of the embryonic leaves are considered to have an important meaning for the well-known function of this organ.

Author wishes to express his gratitude to Prof. T. Mori whose kind aid and advice enabled this study to be accomplished.

Summary

1. The distribution of electric potential on the surface of the seed embryo of *Vigna sesquipedalis* and its changes with the growth stage during 5 days were investigated, using a vacuum tube potentiometer and Zn/ZnSO₄/agar non-polarizing electrodes.

2. Influences of light and mechanical stimuli on the resting potential were also examined, and it was succeeded to exclude these disturbing influences practically by an appropriate device in the measurement.

3. The internal structure of the plant and its changes with growth were explored from the histological aspect in relation to the potential distribution.

4. Experimental results thus obtained indicate that the zonal distribution of the elongation velocity in the axial direction coincides neither with the potential distribution itself as has been hitherto accounted simply nor with that of potential gradient, but most sufficiently with that of the 2nd derivative of the potential, as theoretically warranted in the discussion.

5. A working hypothesis with which these facts are to be explained was based upon an assumption that the distribution of resting potential on the surface of the plant body is determined by the spatial distribution of ionic concentration which ought to refer to the functional differentiation of the tissues concerned.

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Notes on *Lindernia*, *Vandellia*, *Torenia* and their Allied Genera in Eastern Asia II*

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6) Seed formation.

a) Observations on *Lindernia pyxidaria* and *Ilysanthes dubia* (= *Lindernia dubia*). In *Ilysanthes dubia*, the processes of the seed formation quite agree with those of *Lindernia pyxidaria*. The ovary contains numerous small semi-anatropous ovules. The mature embryo sac is spatulate in shape and formed of two portions being the broad globular micropylar and the elongated and nearly straight chalazal ones which are invested by the endothelium (fig. VI, 1). Three antipodal cells are angular and lie side by side. They immediately disintegrate after fertilization. Two polar nuclei meet with each other near the neck of the embryo sac. The egg apparatus occupies the micropylar end of the embryo sac within the ovule. Two synergids are pyriform in shape each containing a large vacuole in its chalazal side. The embryo sac enlarges after fertilization. The first division of the primary endosperm nucleus is followed by a transverse wall to form a micropylar and a chalazal chamber (fig. VI, 2). One subsequent longitudinal division is observed to occur in the micropylar chamber and next in the chalazal one (fig. VI, 3), thus, four celled endosperm is formed. Two chalazal cells do not divide further, but act as two uni-nucleate chalazal haustoria containing dense cytoplasm (figs. VI, 5 and 6). Two micropylar cells undergo a further longitudinal division perpendicularly to the previous plane of division and produce four cells. Transverse divisions in each of these four cells result two tiers of four cells (fig. VI, 4). The micropylar tier does not divide further, but acts as four uni-nucleate micropylar haustoria (figs. VI, 5 and 7). The middle tier continues transverse and longitudinal divisions to produce the endosperm proper, growing rapidly into a ellipsoidal mass of cells (fig. VI, 8). As the embryo develops, the endosperm cells are gradually broken down around the embryo except some outer layers which persist until the seed matures. The endothelial layer disintegrates completely. In the late stage of development of the seed coat, the epidermal cell-walls which still remain as thin membran, break down, thus leaving the reticulate projections being so faint that the testa appears smooth (fig.

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VI, 9). In *Ilysanthes hyssopoides*, Krisina Iyenger (1940 b) reported the similar seed formation. He described that the second division of the endosperm formation is transverse and results in a row of three cells. But in my materials the second division is usually longitudinal.

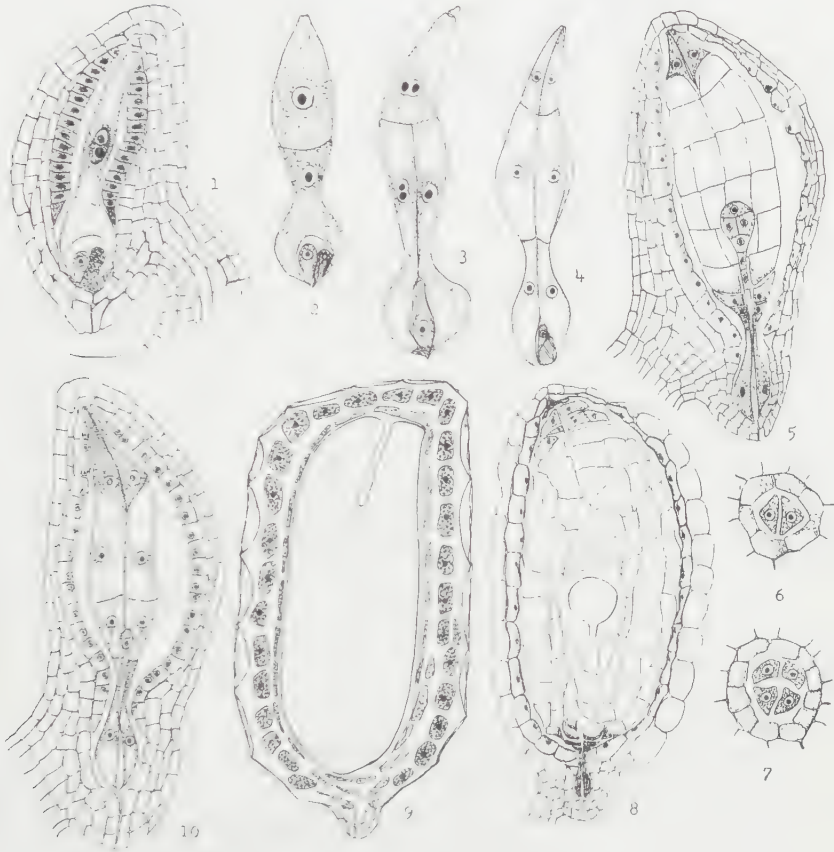


Fig. VI. 1-9, *Ilysanthes dubia* (= *Lindernia dubia*), 1) Longitudinal section of the ovule after fertilization. $\times 640$. 2) Formation of two chambered endosperm. $\times 520$. 3) Longitudinal division of the micropylar chamber resulting three celled endosperm. $\times 510$. 4) Transverse division in each of four micropylar cells results the formation of two tiers of four cells. $\times 490$. 5) Ovule showing the fully developed chalazal and micropylar haustoria. $\times 290$. 6) Transverse section of the two celled chalazal haustorium at the same stage with fig. 5. $\times 400$. 7) Transverse section of the four celled micropylar haustorium at the same stage with fig. 5. $\times 400$. 8) Ovule showing the fully developed endosperm. $\times 240$. 9) Longitudinal section of the mature seed. $\times 220$. 10) *Lindernia pyxidaria*. Longitudinal section of the ovule showing the fully developed two celled chalazal and four celled micropylar haustorium. $\times 490$.

b) Observations on *Bonnaya verbenaeifolia* (= *Vandellia anagallis*) and *Bonnaya ruellioides* (= *Vandellia antipoda*). In *Bonnaya verbenaeifolia*, the ovary contains numerous small semi-campylotropous ovules. The mature embryo sac is spatulate in shape and formed of two portions being the broad globular micropylar and the narrow elongated chalazal ones which is invested by the endothelium and slightly

curves towards the dorsal side (fig. VII, 8). Two polar nuclei meet with each other near the neck of the embryo sac. The egg apparatus occupies the micropylar end of the embryo sac in the ovule. The first division of the primary endosperm nucleus is followed by a transverse wall to form a micropylar and a chalazal chamber. The chalazal cell remains undivided and acts as uni-nucleate chalazal haustorium (figs. VII, 9 and 10). The chalazal portion of this haustorium penetrates into the hypostatical region and extends almost to the epidermis of the ovule, and rapidly forms a bubble containing large vacuole, thus a large gourd-shaped haustorium is formed (figs. VII, 10 and 11). The micropylar cell divides in a longitudinal plane resulting two cells. Each of the two daughter cells of micropylar chamber undergoes a further longitudinal division perpendicularly to the previous plane of division and produces four cells. Transverse division in each of these four cells results two tiers of four cells. The micropylar tier does not divide further, but acts as four uni-nucleate micropylar haustoria (figs. VII, 11 and 12). The middle tier continues transverse and longitudinal divisions to produce the endosperm proper. In *Bonnaya ruellioides*, the processes of the seed formation agree with those of *Bonnaya verbenacifolia*, but the former has the campylotropous ovules and the large conical celled chalazal haustoria (figs. VII, 13 and 14). In *Bonnaya tenuifolia* (= *Vandellia tenuifolia*), Krisina Iyenger (1940 b) reported the similar seed formation. He described that the second division of the endosperm formation is transverse and results in a row of three cells, the chalazal haustorium consists of two uni-nucleate cells which later fuse to form one bi-nucleate haustorium. But in my materials the second division is usually longitudinal, and the chalazal haustorium consists of one uni-nucleate cell from the beginning.

c) Observations on *Vandellia setulosa*. The ovary contains many small anatropous ovules. The mature embryo sac is spatulate in shape, its narrow chalazal portion curves towards the dorsal side. The egg apparatus occupies the micropylar end of the embryo sac in the ovule (fig. VII, 1). The mode of the endosperm formation quite agrees with that of *Bonnaya verbenacifolia* (figs. VII, 2-6). The chalazal haustorium is a large conical cell with a prominent vacuole near the adjacent endosperm cells, dense cytoplasm at the chalazal part and a conspicuous nucleus. The development of the seed coat and the enlarged endothelial cells quite agree with those of *Bonnaya verbenacifolia* and *Vandellia angustifolia*. The mature seed bears some hollows lying in five rows and the projections of thickened radial walls on their surface (fig. VII, 7). In *Vandellia scabra* (= *Vandellia hirta*), Krisina Iyenger (1940 a) reported the similar seed formation. He described that the second division of the endosperm formation is transverse. But in my material it is longitudinal.

d) Observations of *Vandellia angustifolia* and *Vandellia crustacea*. In *Vandellia angustifolia*, the ovary contains many small anatropous ovules being linear in shape. The mature embryo sac is nearly straight. The micropylar part of the embryo sac

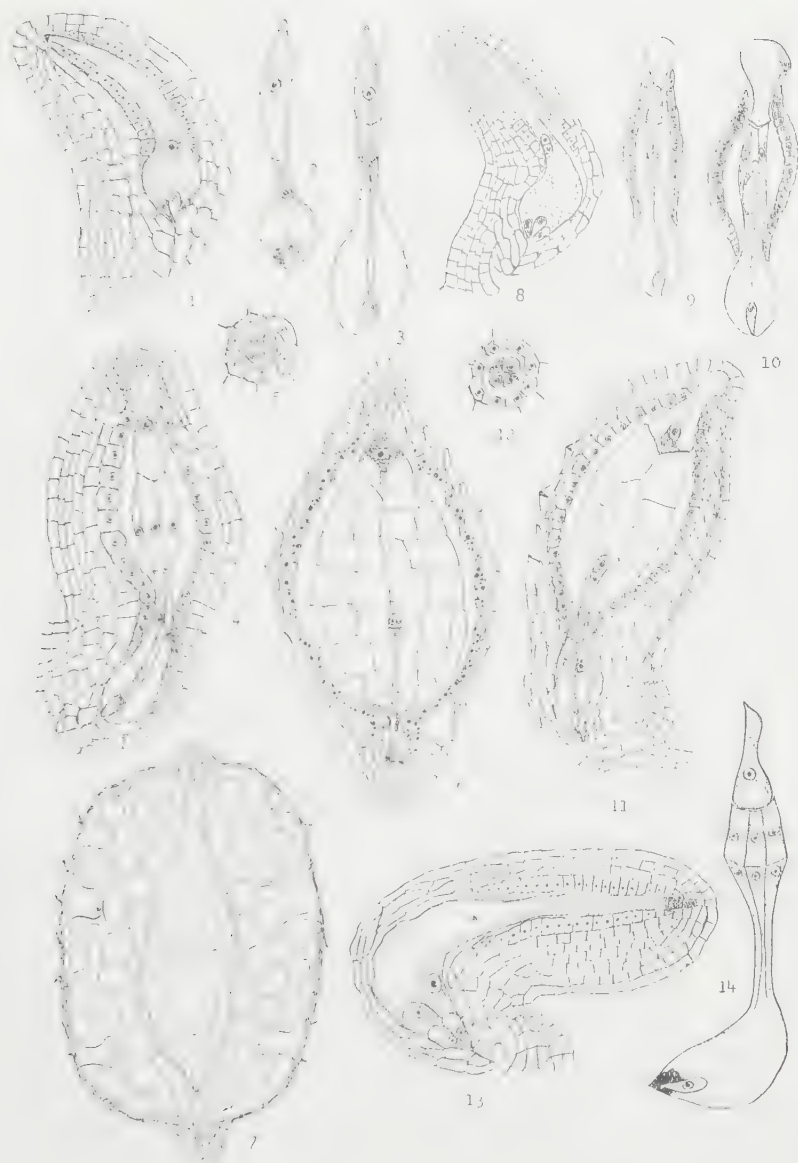


Fig. VII. 1-7, *Vandellia setulosa*. 1) Longitudinal section of the ovule. $\times 360$. 2) Formation of two chambered endosperm. $\times 400$. 3) Longitudinal division of the micropylar chamber results three celled endosperm. $\times 380$. 4) Further stage showing the young chalazal and micropylar haustorium. $\times 340$. 5) Transverse section of the four celled micropylar haustorium at the same stage of fig. 4. $\times 470$. 6) Ovule showing fully developed chalazal and degenerated micropylar haustorium. $\times 240$. 7) Longitudinal section of the immature seed. $\times 190$. 8-12, *Bonnaya verbenaefolia* (= *Vandellia anagallis*). 8) Longitudinal section of the ovule. $\times 310$. 9) Three celled endosperm. $\times 310$. 10) The second longitudinal division of the micropylar chamber results five celled endosperm. $\times 290$. 11) Ovule showing the fully developed chalazal and micropylar haustorium. $\times 230$. 12) Transverse section of the four celled micropylar haustorium at the same stage with fig. 11. $\times 470$. 13-14, *Bonnaya ruellioides* (= *Vandellia antipoda*). 13) Longitudinal section of the ovule. $\times 340$. 14) Further stage showing the young chalazal and micropylar haustorium. $\times 260$.

grows through the micropyle along the funiculus. The egg apparatus is located in the extra-micropylar portion of the embryo sac and directly contacts with the funiculus. After the fertilization the micropylar part of the embryo sac begins to enlarge and contains a large vacuole. The first division of the primary endosperm nucleus takes place with a transverse wall to form a micropylar and a chalazal chamber. The chalazal cell remains undivided and acts as a uninucleate chalazal haustorium (figs. VIII, 2 and 3). The micropylar cell divides on a longitudinal plane (fig. VIII, 2). Each of two daughter cells of micropylar chamber undergoes a further transverse division which results in two tiers of two cells. In each of thus formed two micropylar cells the nucleus divides to form the bi-nucleate cell which functions as haustorium without further cell divisions (fig. VIII, 3). The single chalazal haustorium is a large conical cell with a prominent vacuole near the adjacent endosperm cells, denser cytoplasm near the chalazal part and a conspicuous nucleus. This haustorium penetrates into the hypostatical region and extends almost to the epidermis. As the seed approaches maturity the chalazal haustorium becomes a dark, shriveled structure in the chalazal knob of the ovule. The micropylar haustorium extends through the micropyle and along the funiculus, shrinks somewhat as it becomes older, and persists in the mature seed protruding slightly beyond the seed coat (fig. VIII, 4). In early stage, the undivided and unelongated zygote is surrounded by the micropylar haustorium. When the endosperm consists of many cells, the zygote penetrates into the tiers of endosperm cells.

Very early in embryo development fewer integumentary cell layers are visible between the endothelial layer and epidermis. At the later stage these cells break down, and a few remains of crushed cell can be seen. At the time when endosperm cells are few the endothelial cells are characterized by large appearance and denser cytoplasm (fig. VIII, 3), and many of such endothelial cells, at the later stage, break down leaving few cells which become greatly enlarged and project into endosperm tissue. Thus the enlarged endothelial cells form pockets on the surface of the mature seed (figs. VIII, 5-7). Toward the late stage of development of the seed coat, the radial walls of some epidermal cells become thick and show X-shape in external view. All of the epidermal cells break down leaving the radial thickening and cutinized walls. Thus, the papillae remain of cutinized walls persist on the surface of the mature seed (fig. VIII, 4).

In *Vandellia crustacea*, the ovary contains many small campylotropous ovules being ellipsoid in shape (fig. VIII, 8). The mature embryo sac bends toward the region of the micropyle where the polar nuclei meet with each other. The egg apparatus is located in the extra-micropylar portion of embryo sac and comes into direct contact with the funiculus. The processes of the endosperm formation almost agree with those of *Vandellia angustifolia* in which single uninucleate chalazal and two bi-nucleate micropylar haustoria are formed. A chalazal haustorium contains two large vacuoles in lateral side and denser cytoplasm with a large

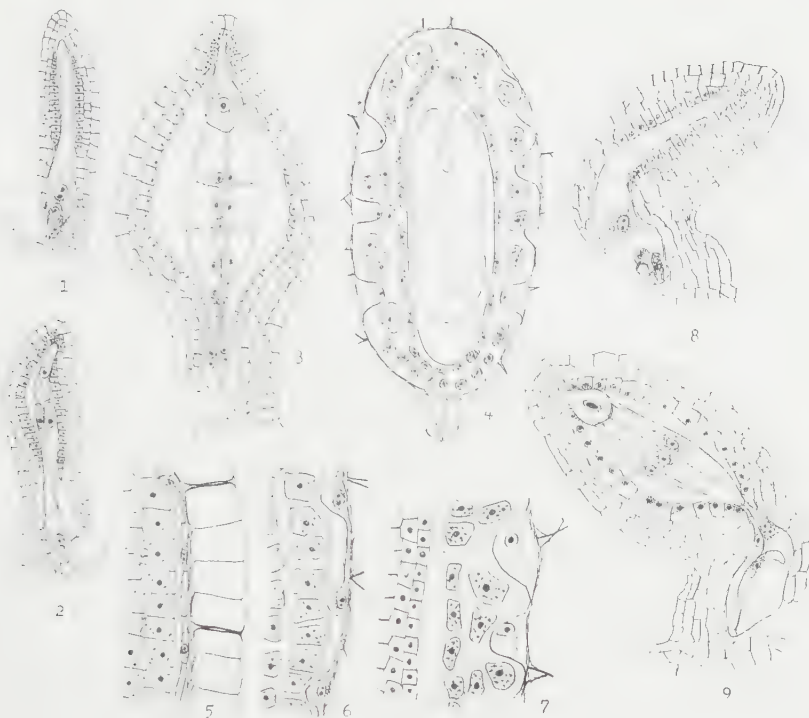


Fig. VIII. 1-7, *Vandellia angustifolia*. 1) Longitudinal section of the ovule. $\times 320$. 2) Formation of three chambered endosperm. $\times 270$. 3) Showing the fully developed chalazal and micropylar haustorium. $\times 310$. 4) Longitudinal section of the mature seed. $\times 190$. 5) Longitudinal section of the young seed showing crushed endothelial cells. 6) The same at the later stage showing few cells which become enlarged and project into the endosperm tissue. 7) Showing the endothelial cells form pockets into the endosperm. 8-9, *Vandellia crustacea*. 8) Longitudinal section of the ovule after fertilization. $\times 290$. 9) Ovule showing the fully developed chalazal and micropylar haustorium. $\times 290$.

nucleus in the middle (fig. VIII, 9). As the embryo begins to divide, two micropylar haustoria merge into a four-nucleate body with a vacuolate cytoplasm extending through the micropyle along the funiculus. The development of the seed coat and the enlarged endothelial cells quite agree with those of *Vandellia angustifolia*. In *Vandellia crustacea*, Srinivasan (1940) reported the similar seed formation. He described that the chalazal haustorium consists of two uni-nucleate cells which later often fuse to form one uni-nucleate cell, and the micropylar haustorium consists of four uni-nucleate cells which later fuse to form one four-nucleate cell. But in my investigation the chalazal haustorium consists of one uni-nucleate cell from the beginning, and the micropylar haustorium consists of two bi-nucleate cells which later fuse to form one four-nucleate cell.

e) Observations on *Torenia violacea*. The oblong and oblique ovary contains many small campylotropous ovules being ellipsoid in shape. A hypodermal arcesporial cell undergoes meiosis and forms the tetrad, the chalazal one functions as the

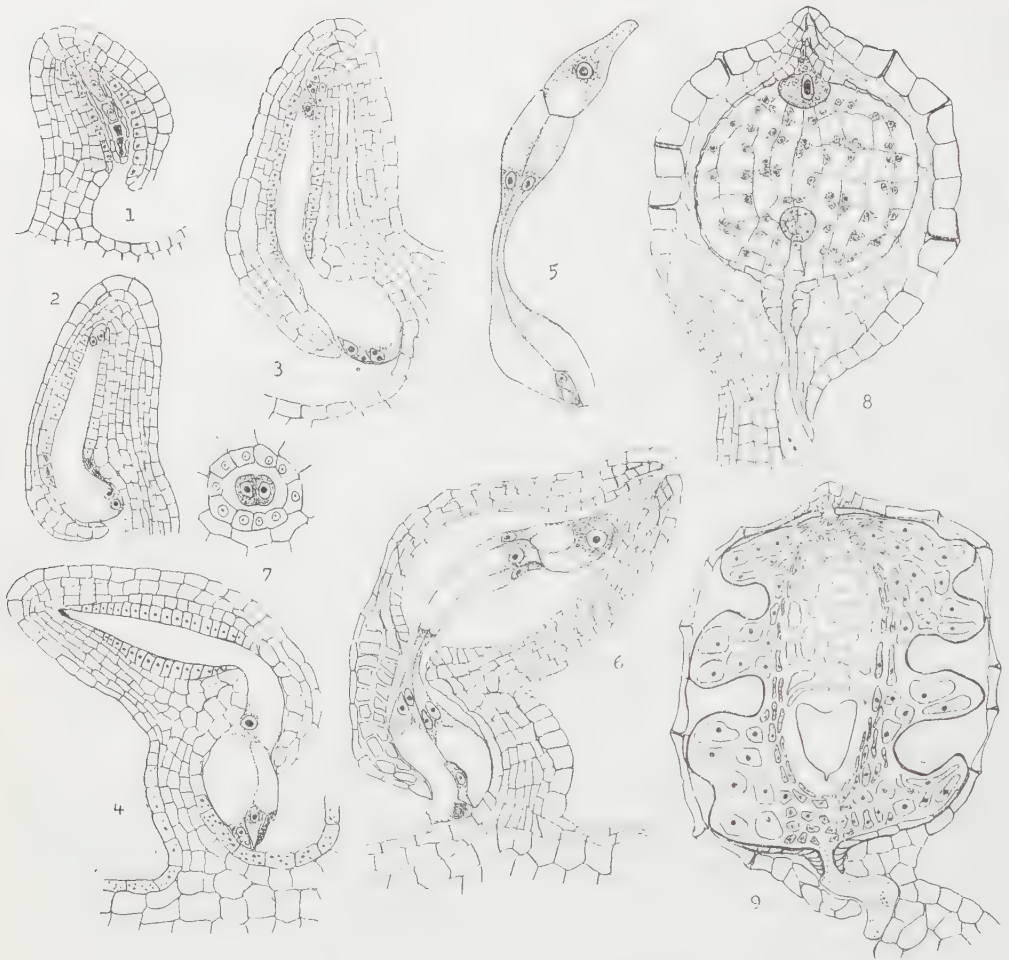


Fig. IX. *Torenia violacea*. 1) Tetrad showing the upper three megaspores degenerating. $\times 400$. Ovule showing four-nucleate embryo sac. $\times 340$. 3) Ovule showing eight-nucleate embryo sac. $\times 400$. 4) Ovule showing the mature embryo sac. $\times 330$. 5) Three celled endosperm. $\times 350$. 6) Ovule showing the fully developed chalazal and micropylar haustorium. $\times 330$. 7) Transverse section of the two celled micropylar haustorium. 8) Young seed showing fully developed chalazal and degenerated micropylar haustorium. $\times 170$. 9) Longitudinal section of the immature seed. $\times 120$.

megaspore mother cell (fig. IX, 1). The megagametophyte grows considerably in length at the two nucleate stage, two daughter nuclei move apart to opposite poles, most of the cytoplasm aggregates around them, the center is occupied by a large vacuole. The next division gives rise to a four-nucleate stage which is followed by the eight-nucleate stage comprising a micropylar and a chalazal quartet (figs. IX, 2 and 3). At the time of eight-nucleate stage, the micropylar part of the naked embryo sac protrudes out of the ovule through the micropyle along the funiculus (fig. IX, 4). Of the four nuclei at the chalazal end, small three give rise to the antipodal cells. The remaining large one moves to the center and gives rise to a

polar nucleus. A narrow mature embryo sac with a bent in the region of the polar nuclei is formed and its chalazal end is narrow while the micropylar end has a large bubble in which the egg apparatus is folded (fig. IX, 5). The fused polar nucleus being surrounded by the dense cytoplasm, is located near the upper end of the bubble. The three antipodals are small and soon disintegrate. As early as the megaspore stage, the nucellar cells surrounding the sac become flattened. At about the time megagametophyte reaches four-nucleate stage, the nucellar cells surrounding the sac are disintegrated completely, and when reaches eight-nucleate stage an endothelial layer of regularly arranged cells is formed from the inner epidermis of the integument. The integument grows considerably in thickness at the dorsal micropylar part, so the ovules change from an anatropous form to a campylotropous. The processes of the seed formation are quite agree with those of *Vandellia angustifolia* (figs. IX, 5-9). Guilford and Fisk (1952) reported the similar seed formation in *Torenia fournieri*. They described that the micropylar haustorium consists of four uni-nucleate cells which later fuse to form one four-nucleate cell. But in my materials the micropylar haustorium consists of two bi-nucleate cells which later fuse to form one four-nucleate cell.

f) The modes of the seed formation of these species. In *Lindernia pyxidaria* and *Ilysanthes dubia* the ovules are semi-anatropous, in *Vandellia setulosa* and *Vandellia angustifolia* they are anatropous, in *Bonnaya verbenaeifolia* it is semi-campylotropous, in *Bonnaya ruellioides*, *Vandellia crustacea* and *Torenia violacea* they are campylotropous. It has been proved from the ontogenetical evidences of many species of Scrophulariaceae that the anatropous ovule may be induced by the semi-anatropous ovule, and also that the campylotropous ovule from the anatropous one, thus the semi-anatropous ovule is considered the most primitive.

In *Lindernia pyxidaria*, *Ilysanthes dubia*, *Bonnaya verbenaeifolia* and *Vandellia setulosa*, the egg apparatus occupies the micropylar end in the ovule, while in *Vandellia angustifolia*, *Vandellia crustacea* and *Torenia violacea*, the egg apparatus goes out of the micropyle prior to fertilization. The bending form and extra-micropylar embryo sac of *Vandellia crustacea* and *Torenia violacea* may be connected with the straight and intra-micropylar one of *Vandellia setulosa* by the intermediate form of *Vandellia angustifolia*.

Lindernia pyxidaria, *Ilysanthes dubia* and *Vandellia setulosa* bear four uni-nucleate micropylar haustorial cells. In *Vandellia angustifolia*, *Vandellia crustacea* and *Torenia violacea* the micropylar haustoria are characterized by having two bi-nucleate cells which fuse to form a four-nucleate haustorium in later stage.

From the above mentioned facts, I can recognize three types of the haustorium in these species. *Lindernia pyxidaria* and *Ilysanthes dubia* belonging the first type, are characterized by the uni-nucleate two celled chalazal and the uni-nucleate four celled micropylar haustorium. *Bonnaya verbenaeifolia*, *Bonnaya ruellioides* and *Vandellia setulosa* belonging the second type, are characterized by the single uni-

nucleate chalazal and the uni-nucleate four celled micropylar one. *Vandellia angustifolia*, *Vandellia crustacea* and *Torenia violacea* belonging the third type, are characterized by the single uni-nucleate chalazal and the bi-nucleate two celled micropylar one. In the modes of the haustorial development of Scrophulariaceae, L. M. Glišić (1937) suggested that the most primitive haustorium comprises four uni-nucleate cells, and two uni- or bi-nucleate celled haustorium and single four-, bi- or uni-nucleate one are to be led from this primitive form. In *Vandellia hirsuta*, Krisina Iyenger (1940 a) reported that the chalazal haustorium consists of two uni-nucleate cells, but the separating membran between the two cells is very thin, so it frequently dissolves away resulting in the formation of a single bi-nucleate haustorium, and one of the nuclei of this haustorium shows a marked tendency to degenerate, which the other enlarges considerably. Some of the sections failed to show any nuclear division in the chalazal chamber, which may thus be uni-nucleate from the beginning. These facts indicate a tendency towards the establishment of a uni-nucleate haustorium. In my present study, I also found that the first *Lindernia* type is the most primitive and the third *Torenia* type is the most advanced one (fig. X).

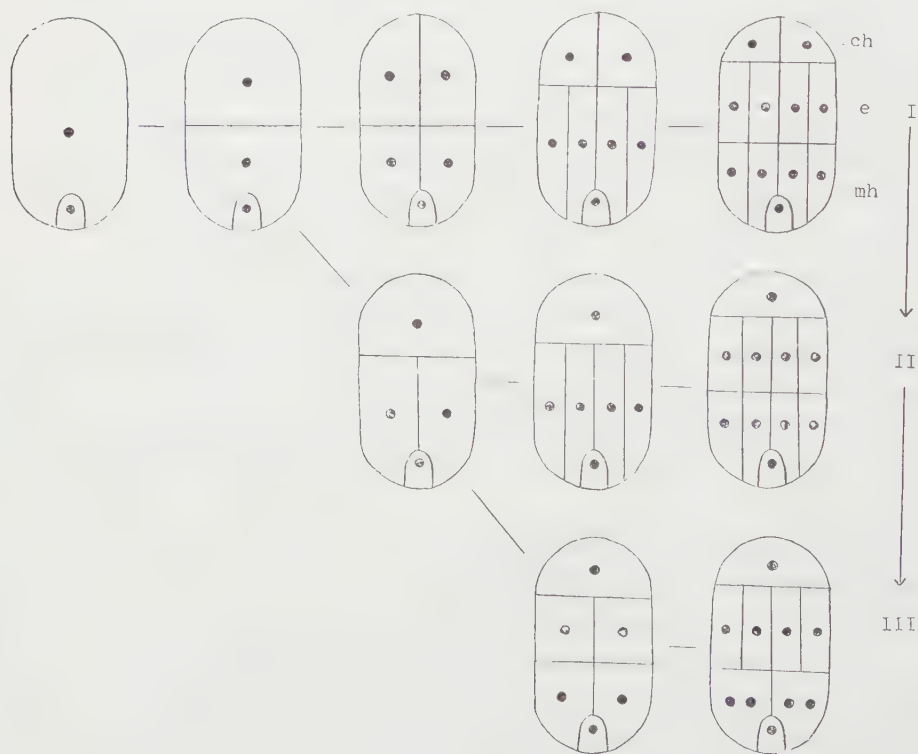


Fig. X. The modes of the endosperm formation. I, *Lindernia* type. II, *Bonneya* type. III, *Torenia* type. ch, chalazal haustorium; e, endosperm; mh, micropylar haustorium.

Table 1

	<i>Lindernia pyxidaria</i> →	<i>Uysanthes dubia</i> →	<i>Vandellia setulosa</i> →	<i>Bonnaya verbenae-fo lia</i> →	<i>Vandellia angustifolia</i> →	<i>Vandellia crustacea</i> →	<i>Torenia violacea</i>
Stamens	4 fertile	2 fertile	4 fertile	2 fertile	4 fertile	"	"
Anthers	emarginate	"	acute	"	"	"	"
Leaf venation	palmate nerves	"	pinnate nerves	"	"	"	"
Ovary	symmetrical	a little oblique	"	"	"	oblique	oblique
Ovule	glabrous	"	"	"	"	"	hairs
	semi- anatropous	"	anatropous	semi- campylotropous	anatropous	campylotropous	"
Embryo sac	intra- micropylar	"	"	"	extra- micropylar	"	"
Micropylar haustorium	uninucleate four cells	"	"	"	bi-nucleate two cells	"	"
Chalazal haustorium	uninucleate two cells	"	single uninucleate cells	"	"	"	"
Seed	smooth surface	"	projected surface	"	"	"	"
	no hollows	"	hollows	"	"	"	"

In *Lindernia pyxidaria* and *Ilysanthes dubia*, as the endosperm full developed, the endothelial cells collapse and disintegrate completely. In the late stage of development of the seed coat, the epidermal cell-walls being composed of thin membran, break down, so the testa appears smooth. In *Bonnaya*, *Vandellia* and *Torenia*, some endothelial cells enlarge markedly and persist in the mature seed as large cells which penetrate into endosperm to form pockets in it. The lateral walls of some outer epidermal cells of the integument bear thickenings. In the mature seed coat these thickenings are left as the projections extend out from the seed.

The above mentioned facts which are listed in Table 1, suggest that *Lindernia* agrees with *Ilysanthes* but remarkably separates itself from others, and maintains the most primitive form. *Ilysanthes* with 2 fertile stamens and 2 staminodes is derived from 4 fertile stamens of *Lindernia*. *Vandellia crustacea* and *Torenia violacea* have the most progressive form as campylotropous ovules, extra-micropylar embryo sacs and bi-nucleate two micropylar haustoria. *Bonnaya* with 2 fertile stamens and 2 staminodes and campylotropous ovules may be derived from the species with 4 fertile stam and anatropous ovules as seen in *Vandellia*.

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単一植物細胞の電気的特性に関する研究 II

滲透現象に伴う原形質膜及び細胞液の抵抗の変化について*

岸 本 卯 一 郎**

Uichirô KISHIMOTO: Studies on the Electrical Properties of a Single Plant Cell II
Changes in the Resistance of Protoplasmic Membrane and of Cell Sap
Accompanying Osmosis

1954 年 9 月 30 日受付

第 1 報において、フラスコの節間細胞の細胞液の抵抗 (r_2 オーム) と原形質膜の抵抗 (r オーム) の変化が、インピーダンスの測定から同時に計算できることを示した。即ち全体のインピーダンスを、 $Z = Z_R - j Z_X$ とすると、細胞液の抵抗の変化については、

$$\frac{\Delta r_2}{r_2} = \frac{\Delta Z_R}{Z_R}$$

の関係があり、原形質膜の抵抗については、

$$\frac{\Delta r}{r} = \frac{1}{2 - \omega C_3 Z_X} \cdot \frac{\Delta Z_X}{Z_X}$$

の関係がある。この式は通常近似的に

$$\frac{\Delta r}{r} = 0.74 \frac{\Delta Z_X}{Z_X}$$

で表わされるが、 Z_X が非常に大きくなつたときは、 $\Delta Z_X / Z_X$ の係数は更に大きい値となる。

本研究においては、筆者が導いたこれらの関係式を用いて滲透現象に伴う原形質膜及び細胞液の抵抗の変化を調べた。滲透現象に関しては既に多くの実験がなされているが、この現象と原形質膜の透過性との関連については確実なデータが少い。上述の方法によれば、細胞になんら害作用を与えることなく、連続的に原形質膜のイオン透過性の変化と、細胞液の電気伝導度、つまりイオン濃度の変化を同時に知ることができる。

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実 験 方 法

実験はヒメフラスモ (*Nitella flexilis*)** の節間細胞を材料として、これを Fig. 1 に示すような 3 つのプールをもつパラフィン製の容器に入れて行つた。プール A, B, C には通常 10^{-2} M KCl



Fig. 1. A vessel used for the impedance measurement.

水溶液を充たし、更に A 又は B, C に適量の蔗糖を添加し、そのときに現れる B, C 間のインピーダンスの変化を第 1 報にのべたようにホイートストン橋によつて測定した。用いた周波数は 1000 c/s である。

実験結果及び考察

一つの細胞の異つた部分が上述のようにそれぞれ異つた滲透圧をもつ溶液に接すると、細胞内に水の移動が起ることが Osterhout (1949), 神谷, 田沢, 黒田 (1952) によつて報告されたが、細胞から水が出る場合と、細胞に水が入る場合とで原形質膜の抵抗がいかに変化するかを調べるために本実験を行つた。実験の順序を外液の組成によつて示すと次の通りである。

A		B と C	
I.	10^{-2} M KCl		10^{-2} M KCl
II.	10^{-2} M KCl + n M Sucrose		10^{-2} M KCl
III.	10^{-2} M KCl		10^{-2} M KCl
IV.	10^{-2} M KCl	10^{-2} M KCl + n M Sucrose	
V.	10^{-2} M KCl		10^{-2} M KCl

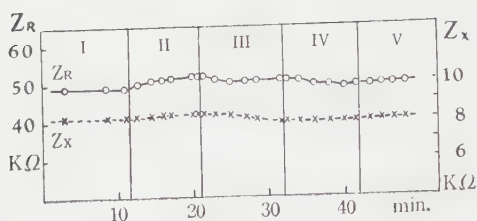


Fig. 2. a

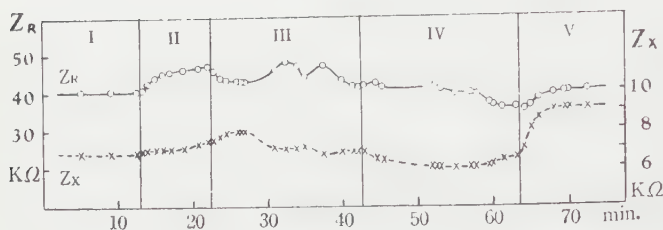


Fig. 2. b

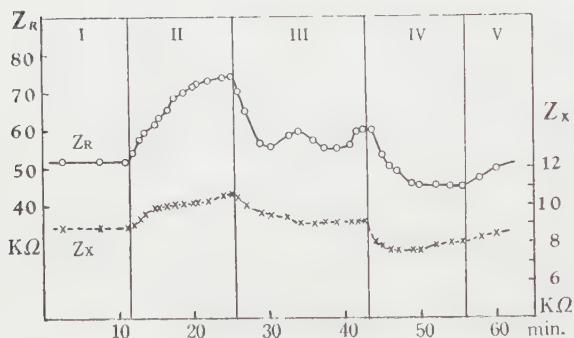


Fig. 2. c

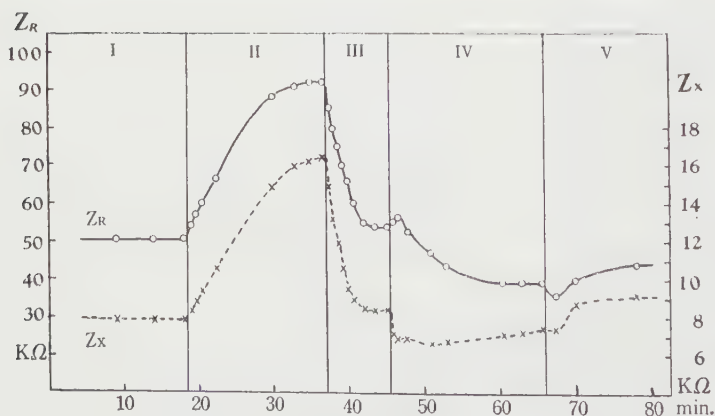


Fig. 2. d

この過程で示されているように、白金電極が設けられているプール B, C は常に同一成分、同一濃度の溶液をもつて充されている。このような処理によつて細胞から水が出る場合と細胞に水が入る場合にかかる原形質膜と細胞液の抵抗の変化を測定することができる。各過程について順を追つて説明すると次のようになる。

I. A と B, C 間に浸透圧の差のないときは細胞内においては水の移動は起らず、従つて勿論 Z_R , Z_X は変化しない。この定常性は非常によく、少くとも8時間以上は保たれる。

II. A 側の外液の浸透圧を B, C 側のそれより大きくすると、この細胞の両半における吸水力の差によつて水は B, C 側より細胞内に入り A 側より出てゆく。このとき水の移動に伴つて細胞液内では溶質の移動が起り、A と B, C 側の細胞の吸水力が等しくなるに到つて水の移動は止み定常状態となる。この場合は水が原形質膜を通つて細胞内へ入る過程のインピーダンスを測定している。

III. B, C 側の外液を元の 10^{-2} M KCl 水溶液に戻すと、II に於て細胞液内でできていたイオン又は溶質の濃度勾配が細胞の両半に前と反対の向に吸水力の差を生ぜしめる。従つて水は A 側より細胞内に入り B, C 側より出てゆく。水の移動

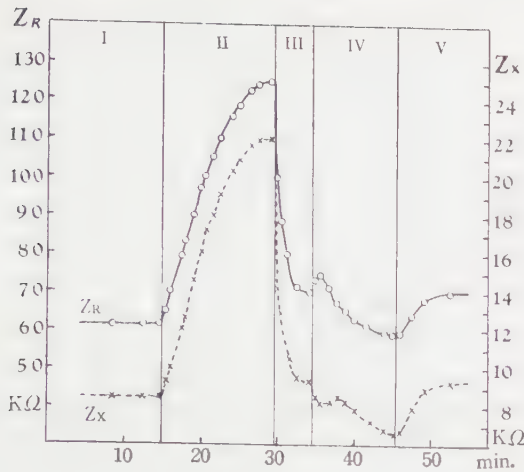


Fig. 2. e

Fig. 2. Time courses of changes in the impedance ($Z = Z_R - jZ_X$) during osmosis. a, b, c, d, and e show the cases of the osmotic gradients of 0.1, 0.2, 0.3, 0.4, and 0.5 Mol respectively. Numbers I-V correspond to the processes explained in the text. No osmosis occurs in I. End-osmosis occurs in II and V. Exosmosis occurs in III and IV. At 25°C.

は細胞の両半における吸水力の差が無くなった所で止む。この場合は水が細胞より出てゆく過程のインピーダンスを測定している。

IV. 次に A はそのままにして、B, C 側の外液の滲透圧を高めると、丁度 II と逆の条件になつて、水が細胞より出てゆく過程のインピーダンスを測定することになる。

V. 最後に B, C 側の外液をもとの 10^{-2} M KCl に戻すと、IV においてできていた細胞液内のイオン、溶質の濃度勾配によつて水は B, C 側より細胞内に入り A 側より出てゆく。この場合は III と逆の条件にあつて水が細胞内に入る過程のインピーダンスを測定している。

蔗糖溶液の濃度を 0.1, 0.2, 0.3, 0.4, 0.5 M の 5 段階に変え、各々の場合について以上の実験を行つた結果を Fig. 2. に示す。これを見ると、細胞より水が出る場合には Z_R, Z_X 共に減少し、細胞内に水が入る場合にはこれらは共に増加している。これらの過程において、細胞内の滲透圧が局部的に変わると原形質流動も一時乱されるが、外液の滲透勾配をとり去れば次第に元の正常な流動

を回復するようになる。

以上の過程における水の移動量、移動速度に関しては、神谷、田沢、黒田 (1952) によつて詳細な実験、計算がなされており、水の移動に伴う溶質の移動に関しては、神谷、黒田の実験 (1953) があり、また滲透に伴う電位差変化については西崎の報告 (1954) がある。第 3 図に示すようにこの細胞を 2 つに仕切り (A 側: l_1 cm, B 側: l_2 cm), A 側の外液の滲透圧を B 側に比べて n モル高くすると、前にのべたように水は B 側より細胞内に入り A 側より出てゆく。



Fig. 3.

この水の移動に伴つて、細胞内では溶質、イオンは A 側に運ばれ、両半の細胞の吸水力が等しくなれば水の移動は止み定常状態となる。この時迄に B 側で x モルの溶質の減少があつたものとするれば、定常状態においては次の条件が成立する。

$$(0.26 - x) - 0.01 = (0.26 + \frac{l_2}{l_1}x) - (0.01 + n)$$

こゝに示した 0.26 なる値は細胞内部の元の滲透圧であり、蔗糖水溶液を用いて原形質分離の方法によつて決定したもので、個体による差は極めて少い。この式より x を求めると、

$$x = \frac{n}{1 + \frac{l_2}{l_1}}$$

となる。この式を用い第 2 図に示した各々の場合についての細胞液内の溶質の移動量、従つてイオン濃度の変化の割合を計算すると、表 I に示すようになる。

細胞内へ水が入る過程 (II) について、細胞液中の溶質或はイオンの濃度変化の計算値 (第 1 表) と、原形質膜及び細胞液の電気抵抗の変化の実測値 (Fig. 2.) との関係を示すと、Fig. 4. のように

表 I

滲透勾配	A 側の細胞の長さ l_1	B 側の細胞の長さ l_2	B 側の細胞液内の 溶質の濃度変化
0.1 モル	11.0 mm	15.0 mm	16.3 %
0.2 "	8.5 "	16.0 "	26.7 "
0.3 "	19.0 "	24.0 "	51.0 "
0.4 "	15.0 "	14.5 "	79.6 "
0.5 "	17.0 "	17.0 "	96.4 "

なる。この図より細胞液の電気抵抗が、細胞液中の溶質或はイオンの濃度に比例して変化していることが判る。この結果は当然予想されることであるが、ここに注目すべきことは、この直線が原点

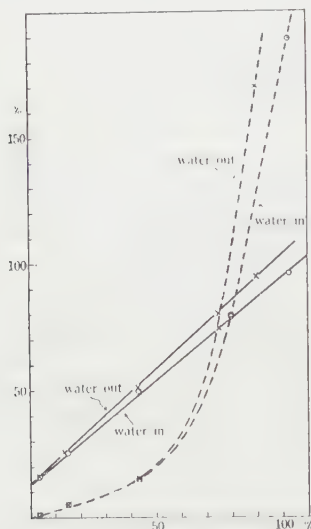


Fig. 4. Relation between the changes in the resistance of the cell sap (measured) and of the ionic concentration in the cell sap. The relation between the changes in the resistances of the protoplasmic membrane and of the cell sap is also shown. — Concentration decrease (calculated) of cell sap on B side (in per cent). Resistance increase of protoplasmic membrane on B side (in per cent).

を通らないことである。これは外液の滲透勾配が小さいときには、細胞液内のイオンの濃度変化が原形質流動による攪拌作用のため減殺され、未だ完全に平衡に達しておらず、従つて電気抵抗が計

算より予想される程には変化しないことによるものと考えられる。外液の滲透勾配が大きくなり、細胞液内の溶質或はイオンの濃度変化が大きくなると、定常状態が速く達成されるようになり、計算値と実測値とは良く一致する。

Fig. 4. に示されているように細胞液のイオン濃度の変化が小さいときには、原形質膜の電気抵抗の変化も小さいが、前者の大きいときには後者は非常に大きくなる。即ち簡単な直線関係ではなく、図にみられるような原点を通り下に凸である一つの曲線で表わされることは興味深い。

再び Fig. 2. にもどると、細胞より水が出てゆく過程 (III, IV) において一時的な原形質膜の抵抗の減少、細胞液の抵抗の増加が現れており、これは一時的に細胞よりイオンの leakage があつたことを示している。即ち滲透勾配の小さいときには、この leakage の現象を数回繰り返して定常状態に達するのがみられる。(Fig. 2. b, c, III) これに伴つて原形質膜の抵抗が一種の減衰振動的な変化をしている。滲透勾配が大きくなると、III の過程が速やかに起るためこの種の変化ははつきりとは観察できないが、滲透勾配の小さいときに著しくなかつた IV の過程の初期の leakage の現象が明瞭に現れる。(Fig. 2. d, e, IV) 外液をもとの溶液にもどしたとき抵抗値がもとの値にもどらなかつたのは、このイオンの leakage にもとづくものと考えられる。

働作流を生ずる場合には、一時的にイオンの leakage があるが、一般に直ちに回復してもとの状態に戻る。しかしこの実験のようにイオンの leakage を起させる原因が相当長時間作用しているときには、細胞に対して多少とも有害な影響を与える。(II) の過程において水が細胞内に入るとつて原形質膜の抵抗は増加し、一応定常状態

に達するが、更に長時間観察しているとやがて次第に減少するようになる、しかもこの現象は浸透勾配の大きいとき程著しく現れる、これは水の出る側で起つたイオンの leakage の現象が細胞に対して有害な作用を及ぼし、この影響が次第に水の入る側にも及んで来たことによると考えられる。

要 約

フラスモ (*Nitella flexilis*) の節間細胞を用いて、浸透現象に伴う原形質膜及び細胞液の電気抵抗の変化について調べた。

この細胞を2つの部分に分ち、この間に蔗糖水溶液で浸透勾配を与えると、水は低浸透圧側より細胞に入り、細胞内を流つて高浸透圧側へ移動す

る。これに伴つて、細胞内ではイオン、溶質が高浸透圧側へ運ばれる。このとき細胞液の電気抵抗は低浸透圧側では高く、高浸透圧側では低くなり、且つその変化は運ばれた溶質の量に比例している。原形質膜の抵抗も水の入る側では高くなり、水の出る側では低くなるが、その変化の割合は浸透勾配の小さいときは小さいが、浸透勾配が大きくなると非常に大きく変化する。

細胞へ水が入るときには、原形質膜に対して著しい影響はみられないが、細胞より水が出てゆくときには多少ともイオンの leakage を伴い、この leakage の現象は細胞に対して有害に作用する。

本研究を行うにあつて終始変らぬ指導と鞭撻を戴いた神谷宜郎教授に厚く御礼を申し上げる。

Summary

In the present report changes in the electric resistance of protoplasmic membrane and of cell sap under osmosis were studied.

When one end of the cell of *Nitella* is subjected to a higher osmotic pressure while the other end of the same cell is under a lower osmotic pressure, water passes through the cell from the hypotonic side to the hypertonic side. This process is accompanied with increase in the resistance of cell sap and of protoplasmic membrane at the part of the cell where water enters; their resistances decrease at the other end where water goes out of the cell.

The change in the ionic conductivity of the cell sap coincides very well with the calculated value of the change in the ionic concentration of the cell sap. The change in the resistance of the protoplasmic membrane is very small under lower osmotic gradient, but increases rapidly under higher osmotic gradient.

When water enters the cell, no remarkable effect is detected as to the resistance of the protoplasmic membrane. On the contrary, when water goes out of the cell, ions leak more or less out of the cell together with water, which gives unfavorable effects on the protoplasmic membrane.

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耐塩菌によるアスパラギン酸の酸化に およぼす阻害剤の影響について

鈴木 米 三*

Yonezo SUZUKI: On the Influence of Inhibitors upon the Oxidation
of Aspartic Acid by *Bacillus pumilus*.

1954 年 10 月 3 日 受附

先に15%の食塩含有培地に培養した *B. pumilus* による二三のアミノ酸の脱水素作用について報告した¹⁾ 中でアスパラギン酸が比較的容易に脱水素されることを見たが、更に数種の阻害剤の影響を実験しアスパラギン酸の酸化に關係する酵素(酵素系?)の性質について考察したのでその結果を報告する。

材料及方法

15%の食塩含有の peptone-broth-agar 培地 (PH 8.2) に 38°C で 48 時間培養した菌体を集め、モスリンで濾過し寒天の細片を除いた後遠心分離により 10%食塩水で数回洗い、30~35°C で 2~3 時間 通気した 菌体の 懸濁液 (resting cell suspension) を菌体標本とした。オクチルアルコール以外の各種阻害剤及 DL- アスパラギン酸ソーダは市販品を再結晶法によつて精製したものをを用いた。

酸素吸収の測定は Warburg 検圧計により、メチレンブルーの脱色時間の測定はツンペリー法によつた²⁾。

結果と考察

i) 青酸塩とオクチルアルコールの影響

第一図は *B. pumilus* がアスパラギン酸を酸化する際の酸素吸収におよぼす青酸塩とオクチルアル

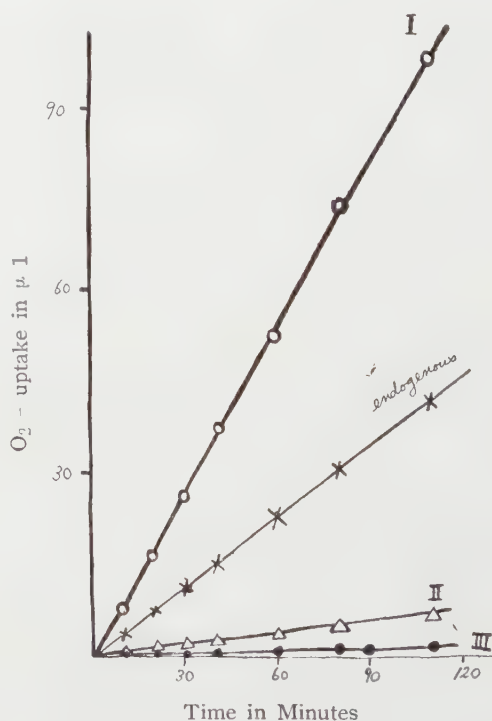


Fig. 1. The effect of cyanide and octyl-alcohol in the oxidation of aspartic acid by *B. pumilus*.

Warburg vessels contained 2.0 ml. of bacterial suspension, 1.0 ml. of M/15 phosphate buffer (pH8.0), 0.5 ml. of 0.1 M. DL-aspartate and 0.5 ml. of inhibitors, and final volume was 4.0 ml.

Curve I. Aspartate only., Curve II. Aspartate and 0.5 ml. of M/100 cyanide. Curve III. Aspartate and 0.5 ml. of saturate octyl-alcohol. 0.3 ml. of 10% KOH in center wall. Gas phase was air 30°C.

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** ピロリン酸塩は生細胞では透過しにくいと云はれているので酵素の阻害が出来ないのかも知れない。又青酸塩の如く促進作用の見られるのは阻害的に働く金属イオンの除去によるものと思はれる⁶⁾。

Table I Effect of concentration of cyanide and azide in the oxidation of aspartic acid by *B. pumilus*.

Final concentration (Mol. conc.)	Endogenous	Cyanide		Azide
		1.25×10^{-3}	1.25×10^{-4}	1.25×10^{-2}
Oxygen uptake per 90 min. (μ l)	65.9	15.0	71.0	56.0

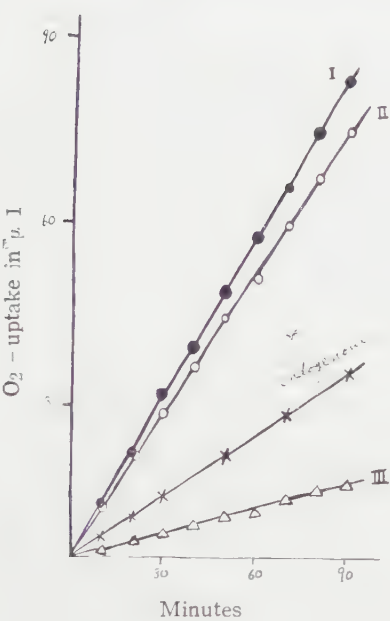


Fig. 2. The effect of arsenate and arsenite in the oxidation of aspartic acid by *B. pumilus*.

Curve I. 1.0 ml. of substrate and 1.0 ml. of M/10 arsenate.
Curve II. substrate only.
Curve III. 1.0 ml. of substrate and 1.0 ml. of M/10 arsenite.

Experimental condition is same to Fig. 1.

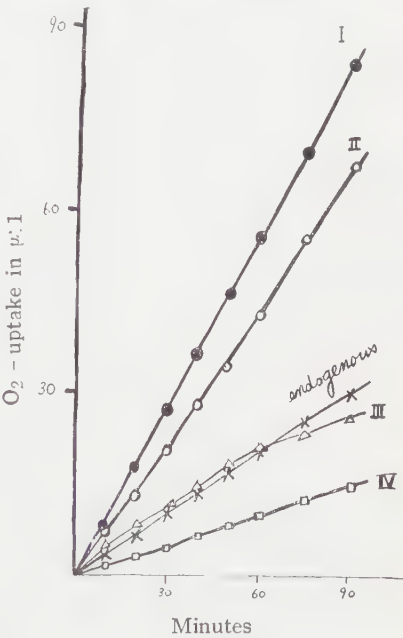


Fig. 3. The effect of ferrocyanide and ferricyanide in the oxidation of aspartic acid by *B. pumilus*.

Curve I. 1.0 ml. of substrate and 1.0 ml. of M/10 ferrocyanide.
Curve II. substrate only.
Curve III. ferrocyanide only.
Curve IV. 1.0 ml. of substrate and 1.0 ml. of M/10 ferricyanide.

Experimental condition is same to Fig. 1.

コールの影響を示す。この結果は Stumpf 等³⁾が *Proteus vulgaris* の L-アミノ酸々化酵素で認めた結果と類似し、両阻害剤とも酸素吸収を著しく阻害するが、第二表に示す様にメチレンプールの脱色はオクチルアルコールでも同様阻害が認められるが青酸塩では阻害は認められず、むしろわずかながら促進的である。又第一表に示す様に青酸塩の終濃度が 1.25×10^{-4} M 程度では酸素吸収も促進

される様な現象が見られる。このことは Stumpf 等が言っている様に青酸塩は基質による酵素の還元反応を阻害するのではなく、基質によつて還元された酵素が酸素分子によつて再酸化される反応を阻害するのであつてこの酸化系が鉄を含む酵素に関係していることを示すものである。ツンベリー法で青酸塩により脱色時間が短縮されることは基質の水素を易動化する酵素が実験の反応系の中

Table II Effect of inhibitors in the dehydrogenation of aspartic acid by *B. pumilus*.

Inhibitors			Final concentration (Mol)	Time of reduction (min.)
Thiol inhibitor	None		10.0—11.0
	Monoiodoacetic acid		0.2×10^{-3}	11.3
	" " "		0.2×10^{-2}	∞
	Mercuric chloride		0.2×10^{-7}	11.3
	" " "		0.2×10^{-5}	22.0
	" " "		0.2×10^{-3}	∞
Carbonyl reagent	Hydroxylamine-HCl		0.2×10^{-4}	17.0
	Hydrazine sulphate		0.07×10^{-3}	14.0
	Semicarbazide-HCl		0.2×10^{-1}	∞
Metal enzyme inhibitor	Cyanide		0.2×10^{-2}	9.3
	Azide		0.2×10^{-3}	15.0
	" "		0.2×10^{-1}	∞
	Pyrophosphate		0.2×10^{-1}	9.3
Narcotics & others	Octyl-alcohol		saturate/5	∞
	Ethyl-urethane		0.2×10^{-2}	12.3
	Chlorate		0.2×10^{-2}	∞
	Benzoate		0.2×10^{-3}	11.0
	" "		0.2×10^{-1}	26.0

Remarks :

- 1. Each Thunberg tube contained 1.0 ml. of cell suspension, 1.0 ml. of M/15 phosphate buffer (pH 8.0), 1.0 ml. of M/10 DL- aspartate solution, 1.0 ml. of methylene blue (1:10,000), and 1.0 ml. of inhibitor. Each final volume was 5.0 ml. 30-31°C
- 2. The standard color was made of 0.1 ml. of methylene blue (1:10,000), 1.0 ml. of heat inactivated cell suspension and 3.9 ml. of dist. water.

Table III Effect of arsenate and arsenite in the dehydrogenation of aspartic acid by *B. pumilus* (Experimental condition is same to Table I.).

Final conc. (Mol)	Time of reduction (min.)		
	endogenous	Arsenate	Arsenite
aspartate only	10—11
0.2×10^{-1}	8.0	40.0
0.2×10^{-2}	10.0	17.0
0.2×10^{-3}	10.0	14.0

に微量に存在すると考えられ得る重金属により阻害され易く青酸塩がこれら重金属と結合して阻害作用を除くためでないかと考えられる（この事は脱水素酵素の多くが SH 基を必要とすることから推察される）⁴⁾。

ii) 各種阻害剤の影響

各種阻害剤の影響について実験した結果を第二

表に示す。これらの結果から、このアミノ酸に係する脱水素酵素が多くの脱水素酵素と同様 SH 基を必要とし（第三図の赤血塩の阻害からも推察される）、又カルボニール基をも必要とすることが分る。Iyer⁵⁾ 等は *Vibrio cholerae* によるアスパラギン酸の酸化的脱アミノが Mg イオンによつて活性化されると報じている。然しピロリン酸ソー

メ**の作用や第三図に示す様に黄血塩の酸素吸収促進などから見てこの内では Mg^{++} イオンが酸化に必要であるかどうか疑問である。又メチレンブルー脱色時のアザイドの阻害が金属イオンに関係しているかどうかも疑問である。チオイソチオチン酸一種であるエチルウレタンによる阻害は著しくはない。

塩素酸塩の添加によつて脱色は強く阻害されるが、このことは塩素酸塩が硝酸塩の如く⁷⁾生体の酸化還元に関係するためとも考えられる。

iii) 砒酸塩と亜砒酸塩の影響

哺乳動物の腎酵素によるアスパラギン酸の酸化が亜砒酸塩によつて強く阻害されることはかつて Krebs⁸⁾によつて報告された。*B. pumilus* のアスパラギン酸々化も第二図、第三表に示す様に亜砒酸塩によつて阻害される。然し砒酸塩はこれに反して促進作用を示す。この促進作用、いくつかの酵素について報告されているが⁹⁾、磷酸カン

ニウ液の存在下での砒酸塩による促進は磷酸塩の代替性として論ずることには疑問があると思われる。

iv) 黄血塩と赤血塩の影響

黄血塩は蛋白質の SH 基を特異的に酸化することから SH 基の定量に利用されているが、このことは又酵素の活性基中の SH 基の測定に用いられている。この菌のアスパラギン酸の酸化は第三図に示す様に赤血塩によつて強く阻害されるが黄血塩の添加は反対に酸素吸収を促進する（然し *E. coli* の L-アスパラギン酸々化では阻害的である）。この促進作用の原因については不明であるが、 Fe^{+++} が阻害的であつて黄血塩が微量に存在する Fe^{+++} を除くためとも考えられるが $\alpha\alpha'$ -デヒリザールの影響から見て Fe^{++} は酸化に必要ではない様である¹⁰⁾。

御教示をいただいた柴田萬年教授に感謝の意を表します。

Summary

1. Aspartic acid was oxidized both anaerobically and aerobically by the resting cell of *Bacillus pumilus*.
2. Cyanide inhibited the oxidation of aspartate aerobically but not anaerobically. This indicates that the observed inhibition is due to the reoxidation of the reduced enzyme by molecular oxygen but not to the reduction of the enzyme by its substrate.
3. Mercuric chloride, monoiodoacetic acid, and ferricyanide inhibited the oxidation activity of bacteria these findings suggest that sulfhydryl group might be involved in the oxidation process.
4. The sensitivity of the oxidation process to hydroxylamine, hydrazine and semicarbazide suggests that active carbonyl groups may also be present in the enzymes.
5. From the reaction with pyrophosphate and ferrocyanide it might be concluded that Mg -ion is not essential in this oxidation process.
6. The mechanism of the acceleration by arsenate and ferrocyanide is obscure.

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編集後記

新年おめでとうございます。昨年の総会できまつたとおり（昨年 11/12 月号大会記事参照）いよいよ本誌も多年懸案の月刊を実施することになりました。毎月 25 日発行ですから遅くとも月末までにはその月の号が手元にとどくことになります。その第 1 号としてこれをお送りしたわけですが、ご覧のとおり紙質もぐんと向上し印刷面も大分見よくなつたものの頁数が少しへりました。これは月刊のため 32 頁建としたのでやむを得ませんがこれでも年間を通ずると今までよりも約 84 頁の増加となり相当収載量がふえることになります。

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Genetical Studies of Plantains* I

Genic Analysis in the Hexaploid and Tetraploid Plants

by Atusi YAMAURA**

山浦 篤: オオバコの遺伝研究 I. 6倍種, 4倍種の遺伝子分析

Received July 8, 1954

Introduction

The plants in the genus *Plantago* found in Japan constitute a 2x-4x-6x polyploid series. On the detailed cytological studies of plantains in our country the writer will report in a later paper of this series. In the present paper he is going to deal with some characters analysed genically of hexaploid plantains, *P. japonica* and *P. japonica* f. *polystachya*, and of the common tetraploid plantain, *P. major* var. *asiatica*.

After Ikeno's studies on the common plantain^{2~7)}, hardly any genetical investigations on this plant have been published, and the plants collected by him seem to have been lost by now. His studies, however, did not go beyond the certification of genes regarding the forms of leaves by means of cross-experiments between the typical and teratological leaves, and the relations among the genes acting in the latter forms left unsolved. Are there any genes of multiple alleles and of linkage groups among the genes responsible for the characters dealt with? The characters of the common plantain studied by Ikeno are as follows:

incisa, *contorta*, *lethal* closely linked with *contorta*, *contracta* and *variegata*.

Even among these characters, there seem yet to remain various characters to be studied in detail. On the diploid plantain accessible here in Tokyo, *P. lanceolata*, the writer will report in a later paper of this series.

Genic Analysis in the Hexaploid Plantain

Single spike vs. *branched* spike: As for the materials of this experiment, *P. japonica*, a normal form with long simple spikes, was obtained by the writer near Tokyo Bay and *P. japonica* f. *polystachya*, a form with branched spikes, was sent to him through the courtesy of Dr. M. Chino.

Cross-experiments, reciprocally performed, gave the normal form in F₁ offspring, and in F₂ a monogenic segregation into the normal form with single spikes and the

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** Tokyo Metropolitan Kitazono High School 東京都立北園高等学校

form with branched spikes in about a 3:1 ratio, as the following Table I shows.

Table I. Segregation in F₂ progeny of *branched* spike × *single*

Phenotype	Single(+)	Branched(b)	Total	
Frequency observed	164	55	219	$\chi^2=0.00152$
Frequency expected in a 3:1 ratio	164.25	54.75	219	$P>0.95$

In the F₂ offspring of this experiment were found plantains in which one or two among the spikes of a stock have only one, two or three branches, though of rare occurrence (less than 2%), while each spike of a polystachous stock has ordinarily 3 to about 40 branches. The plants of this rare form were classified into the group of a single form in the above table.

Whether a plant has *single* or *branched* spikes, will be distinguished before the spikes reach about 5 cm long. According to the writer's counting, frequency of the

spike shapes in the F₂ offspring raised in a definite place showed different results, contrary to expectation; for example, the segregation of 51 *single*: 6 *branched*. This exceptional phenomenon was caused probably by the abnormal humidity in such a place in addition to the rainy weather in the summer of 1953. That *P. japonica* f. *polystachya* may be from time to time variable, some having the form of a sigle spike, was remarked also by Ikeno. He observed in the summer of 1928 that the constant polystachous form of the plantains cultured for long years in his garden came to have almost completely an allelomorphic character, a single spike, owing to the humidity of especially high grade.

Although this change of *polystachya* to the normal single spike form was of frequent occurrence owing to the environmental conditions, the reverse change from genotypically single form to *polystachya* was not observed. Where the writer's material, *P. japonica*, a typical form of single spikes, was obtained, there was not



Fig. 1. Left, a spike of a common tetraploid plantain (ca. 10 cm). Right, that of F₁ plant (ca. 50 cm), of hexaploid plantains *branched* × *single*.

found a plant of branched spikes, *P. japonica* f. *polystachya*.

Genic Analysis in the Tetraploid Common Plantain

a) Pale yellow seedling-cotyledons

The writer found a plant of the common plantain, *P. major* var. *asiatica* which



Fig. 2. *Single* (left two) and *branched* (right two) spikes of hexaploid plantains

by self-pollination segregates the seedlings of normal green and pale yellow cotyledons in a ratio of 3:1, the latter being characterized by a weak chlorophyll development and perishing in two or three weeks after germination.

It is interesting to find through several years' experiences that this almost inviable plant is able to grow to maturity, though very weak, under favorable conditions. The leaves of this plant are conspicuously variegated.

By the cross-experiments the writer recognized in F₂ offspring a 3 *normal*: 1 *yellow* segregation ratio and also a 15 *normal*: 1 *yellow* segregation ratio, as the following Table II shows. From these data two duplicate genes *y*₁ and *y*₂ were assumed.

Table II. Segregation in F₂ offspring of a cross, pale *yellow* seedling-cotyledons × *normal* green

Phenotype	Normal(+) Yellow (<i>y</i> ₁ , <i>y</i> ₂) Total			
Frequency observed	1094	375	1469	$\chi^2=0.232$
Frequency expected in a 3:1 ratio	1102	367	1469	$P>0.50$
Frequency observed	1056	79	1135	$\chi^2=0.962$
Frequency expected in a 15:1 ratio	1064	71	1135	$P>0.30$

b) A strain heterozygous for *variegata* producing an abnormal segregation ratio

The segregation ratios as for chlorophyll deficiencies of the leaf, *variegata*, in the common plantain are expected to be ordinarily 15 *normal*: 1 *variegata* or 3

normal : 1 *variegata*, and the existence of two duplicate genes, *G* and *H*, for the development of chlorophyll was already suggested by Ikeno^{2, 3, 6)}. A genotype *GgHh* may give a 15:1 segregation ratio in the offspring, and *Gghh* and *ggHh* a 3:1 ratio. In the common plantain which is phylogenetically tetraploid, the existence of duplicate genes is expected as a matter of course. The gene *G* will be the same as the gene *H*, and *g* the same as *h*, and also y_1 and y_2 , which were studied by the present writer, will be the same in fact.

A stock of *contorta* form with normal green leaves was found by the writer which segregates in the offspring in an abnormal way as for the leaf color, in about a 1 *normal* : 2 *variegata* ratio, as the table below shows:

	<i>normal</i>	<i>variegata</i>
1951	48	74
1953	69	158

The hybrid plants between this stock and other plants of this species with normal leaves gave a segregation ratio, 15:1. Ikeno^{4, 6)}, studying the common plantain heterozygous for the *contorta* form, observed a strain which segregates quite abnormally. The offspring of his strain always consisted of two types, one *typica*, the other *contorta*, in which the number of *contorta* was usually much greater than that of *typica*, the average ratio being equal to 70:30 %, contrary to the ordinary segregation ratio of 1 *contorta* : 3 *typica*. Such a genetical behavior of the common plantain seems to be of the same type as found by the present writer in the segregation ratio of the *variegata* form.

The *contorta* form is characterized by leaves which wind in an abnormal way either to the right or to the left, and it is ordinarily recessive to *typica*.

This genetical feature was explained by Ikeno on the assumption of an allele, *L* and *l*, which is closely linked with *T* (*typica*) and *t* (*contorta*) respectively, and inhibits partially the action of *T* according to the environmental conditions. When selfed, a heterozygous plant segregates as follows:

$$(TL\ tl)^2 \longrightarrow TL\ TL + tl\ tl + 2TL\ tl,$$

and the first and second terms, *TL TL* and *tl tl*, are not viable, so the offspring consists only of *TL tl* which phenotypically gives either *typica* or *contorta* form, owing to the partially inhibiting action of *L* and *l* on the effect of *T*.

As the abnormal segregation into 2 recessive form vs. 1 dominant form is usually about the same, the present writer can not always agree with Ikeno's hypothesis on the two genes which inhibit partially the formation of dominant trait according to the environmental conditions. The writer's observations on the F_3 offspring, which are now being carried on, will throw light on this abnormal genetic feature.

Résumé

1. Genic analysis concerning the shapes of spikes in the hexaploid plantains

revealed that the branched spike (*b*) is a simple mendelian recessive to the single form. Spikes of *polystachya* are not always branched, but some show single spikes, probably owing to the humidity of specially high grade.

2. In the common tetraploid plantain, pale yellow seedling-cotyledons were found by the writer to be inherited by the recessive duplicate genes (y_1, y_2) to the normal green.

3. A strain heterozygous for *variegata* in the common tetraploid plantain was found, which segregated *normal* and *variegata* in an abnormal ratio of about 1:2, contrary to expectation of an ordinary segregation ratios of 15:1 or 3:1.

The writer wishes to express his cordial thanks to Prof. Shinotô of International Christian University, who gave valuable advices in the course of the present work.

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抄 録

ジャガイモの澱粉粒の縞模様は一定の光と温度の条件下でもつくられる

[Roberts, E. A. and Proter B. E.: The appearance of starch grains of potato tubers of plants grown under constant light and temperature conditions. Science **119**: 509-510 (1954)]

澱粉粒の縞模様は線状とか層状構造などと呼ばれているが、この縞模様は外的条件、即ち光や温度の影響によることを最初 Van de Sande-Bakhuyzen (1926) によつて述べられた。後は光と温度の一定条件下で生育させたコムギでは澱粉粒に縞模様の生じないことを注意した。

著者らはジャガイモ (品種 katahdin) を11月12日から翌年6月3日まで 3,000W のランプで地上 42 in. (約 107 cm) から連続的に照射した。

ランプから 24 in. (約 61 cm) の距離では 0.15 gram-cal/cm² min で 650 フート燭光であつた。室温は 63° ± 2°F (約 17° ± 1°C) で温度は調節されなかつた。他方夏の間に野外で同品種のジャガイモを栽培し、両者の塊茎の澱粉粒約 1,000 個について比較した。

その結果両者とも縞模様をもつていて、ほとんど表面上の差異がなかつた。 (植田利喜造)

Studies on the Germination of Grass Pollen* I

Liquid Exudation of the Pollen on the Stigma before Germination

by Kotaro WATANABE**

渡辺光太郎：禾穀類花粉の発芽に関する研究 I. 柱頭上の花粉における液の滲出

Received November 2, 1954

Generally, grass pollen is regarded to be very difficult of germination on artificial culture media. Many investigators^{1-5, 7)} have tried to induce artificially the germination of grass pollen, and some of them^{8, 9)} used stigmata in the germination tests. However, detailed observations of the germination process on the stigma are lacking almost completely, so far as the present author is aware¹⁾. In the germination process at natural pollination, the interaction between pollen and stigma should be taken into consideration. The effect of the pollen grain on the stigma was examined by Kato⁶⁾.

As to the germination process of the barley pollen, a remarkable observation was made by Anthony and Harlan¹⁾: "Pollen brought in contact with the stigma attaches itself by means of some adhesive substance present, not only on the entire surface of the stigma hairs but also on the pollen grain itself. Normal viable pollen shows a distinct swelling, evidenced by different bulgings of the grain, which gradually disappear." This observation seems to the present author to have nothing to do with the change of shape usually observed and described in the germination process of pollen, such as projection of the pollen tube, swelling, shrinkage of the grain, and so forth. In the present study special attention was given to the morphological changes occurring at the first stage of pollination.

Observations

A mature stigma picked out from a floret was placed on a slide glass, pollinated with pollen from a dehiscing anther, and immediately observed under the microscope. The following description deals mainly with pollen of wheat and barley.

I) Liquid exudation of pollen

a) Change of pollen shape. On the stigma, some of the pollen grains begin to shrink several minutes or later after the pollination. The rest of the grains in most

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** Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University 京都大学農学部応用植物学研究室

cases undergo the following changes and germinate.

In about ten to thirty seconds after the contact of the pollen grains with the stigma, they start to change their form. At first some small protuberances or bulgings appear on their surface. Frequently they emerge from one part of the grain, then appear in another place until the whole surface of the grain is covered with them. But in some cases the bulgings are restricted only to one or to a few parts of the grain. Soon these small bulgings are transformed into large wart-like protuberances by uniting in groups, and this process is repeated until a small number of larger bulgings are formed. Since the first small bulgings do not appear at the same time on the whole grain surface, the formation of the large wart-like bulgings does not occur simultaneously.

After a little while, generally in several seconds, the bulgings disappear and the pollen grain, recovering its smooth surface, returns to the original shape. A difference in the size of a grain before and after the changes above described can hardly be perceived. Pollen grains which rapidly change their form to a great extent are apt to burst. Generally, it takes about thirty to sixty seconds, for the phenomenon of emerging and disappearing of bulgings, and the phenomenon is hardly seen two minutes after pollination. Only in rice and maize, it takes several minutes for the whole process in some cases. Such change of pollen shape on the stigma was noticed hitherto by the author in 28 species, belonging to 19 genera of Gramineae*.

The "wart-like form change" can also be observed in liquid paraffin and on agar or on water surface. In liquid paraffin the change proceeds very slowly so that the process can be observed more accurately.

Almost simultaneously with the appearance of the wart-like bulgings, a small quantity of liquid, apparently colorless, oozes out at the portion of the stigma with which the pollen grain is brought in contact. This exudation was noticed in some cases to precede immediately the beginning of the wart formation in wheat, rye, maize, pearl-barley, etc. The liquid rapidly increases in amount which becomes stationary before the wart-like bulgings disappear. In maize it takes about fifteen to twenty seconds for the complete process. Thus, the pollen grains are forced to stick more tightly to the stigma hairs by the capillary force of the liquid. With the increasing quantity of liquid, the grain attached to the top of a papillate cell of a stigma hair glides slowly or rapidly along its side walls, and frequently jerky movement of the grain can be observed. Sometimes a liquid exudation occurs without any wart-like form change, but in such case, the amount of liquid is mostly small.

The liquid persists for five to six minutes in Einkorn wheat and for ten to twenty minutes in rye. Thereafter some pollen grains remain unchanged and begin

* *Aegilops Aucheri*, *A. caudata*, *A. squarrosa*, *Agropyrum ciliare*, *Avena sativa*, *Coix Lachryma-Jobi*, *C. Ma-yuen*, *Haynaldia villosa*, *Hordeum sativum*, *Melica nutans*, *Microstegia vimineum*, *Oryza sativa*, *Panicum Crus-galli* var. *submutica*, *Pennisetum japonicum*, *P. typhoideum*, *Phalaris arundinacea*, *Poa annua*, *Secale cereale*, *Setaria italica*, *S. lutescens*, *S. viridis*, *Trisetum bifidum*, *Triticale* sp., *Triticum monococcum*, *T. orientale*, *T. Spelta*, *T. vulgare* and *Zea Mays*

to shrink, while the grains which proceed to germination are turgid for a long time. The process of wart formation and liquid exudation in Einkorn wheat and maize is illustrated in Fig. 1 to 8.

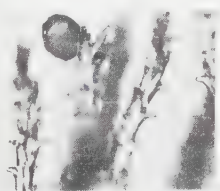


Fig. 1

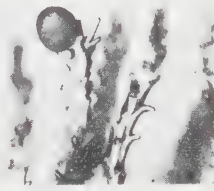


Fig. 2

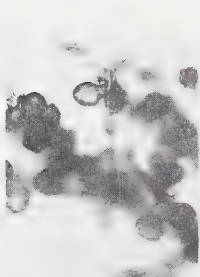


Fig. 3

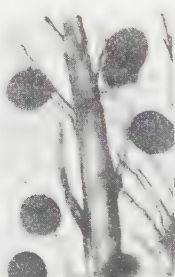


Fig. 4



Fig. 5

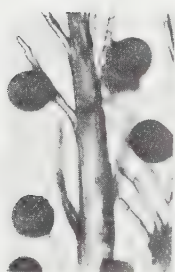


Fig. 6



Fig. 7

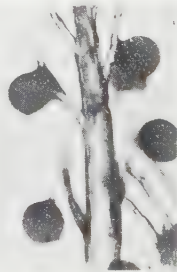


Fig. 8

Fig. 1~3. Change of pollen shape on stigma in *Triticum monococcum*. Fig. 1. 25 seconds after pollination, wart-like protuberances. Fig. 2. 45 seconds after pollination, shows the same grain returned to its original shape. Fig. 3. 24 seconds after pollination, wart-like protuberances.

Fig. 4~8. Process of wart formation and liquid exudation of pollen on stigma in *Zea Mays*. Fig. 4. 55 seconds after pollination: pollen grain A, no change of shape yet; B, beginning of liquid exudation between pollen and stigma. Fig. 5. The same after 2 minutes and 17 seconds: grain A, some small wart-like bulgings; B, increase of the liquid in amount. Fig. 6. After 2 minutes and 29 seconds, and Fig. 7 after 2 minutes and 42 seconds: grain A, larger wart-like protuberances. Fig. 8. 4 minutes and 24 seconds after pollination: grain A returned to its original shape; formation of a nipple-like protrusion.

b) The nature of the "wart-like form change" As mentioned above, the change of shape and the exudation of liquid occur almost simultaneously, or the latter immediately precedes the former. Both phenomena, therefore, seem to have a close relationship and, to elucidate this point, a few experiments with rice were undertaken with the help of microdissecting. The liquid was observed not only on the

surface of the part of the stigma to which the pollen stuck but also in the vicinity. Pollen grains which had finished the wart-like transformation were also covered over the whole surface with a liquid; and if a needle touched a large wart-like protuberance, it collapsed immediately and a droplet of liquid adhered to the needle. Also it was observed that when a pollen grain with the wart-like bulgings came into contact with another grain or a stigma hair, the bulgings fused promptly with each other, and then the liquid spread and filled out the space between the two grains or between the grain and the hair. From these observations the wart-like form change seems to be due to the liquid exudation from the pollen grains probably caused by a rapid increase in permeability.

In one case, a pollen grain was observed to be in contact with two stigma hairs, and thereafter it adhered tightly to one hair and was released from the other. A thread was formed between the grain and the released stigma hair, which remained without any change for a long time. The liquid adhering to the micro-needle dried in the air to a solid mass. It seems, therefore, to be highly viscous.

II) Nipple-like protrusion and germination

In a little while after the pollen grain returns to its original shape, a nipple-like protrusion appears at the germ pore of the grain. By nipple-like protrusion the present author means a protrusion of the cell content. It cannot be regarded to be the pollen tube, for it can be produced in dead pollen grains* when supplied with adequate moisture. The nipple-like protrusion appears usually one to two minutes after pollination. Following this protrusion the grain develops a pollen tube. However, the pollen grains which from the nipple-like protrusion do not always germinate, but sometimes remain in this condition for a while and then begin to shrink. But germination is always preceded by the nipple-like protrusion and by the exudation of liquid mentioned above. That pollen which is unable to germinate never exudes any liquid was ascertained from observations of preserved pollen, sterile pollen and pollen killed by heat or alcohol vapors. So it is sure that there is a very close relationship between the liquid exudation and pollen germination. It was found in rye and wheat that germination occurred even in pollen grains which, without touching the stigma, were in contact with other grains attached to it, and also in such cases a liquid was observed between these grains.

The whole process of the germination of grass pollen can be illustrated by the following schema. As shown in the schema, the germination proceeds in several ways. The most frequently observed course of germination is the following: exudation of liquid—→wart-like form change—→nipple-like protrusion—→germination. When the progress is disturbed for some reason or other, the pollen tends to shrink or burst at any stage.

* Rye pollen killed by heat, or preserved for 1 to 2 years

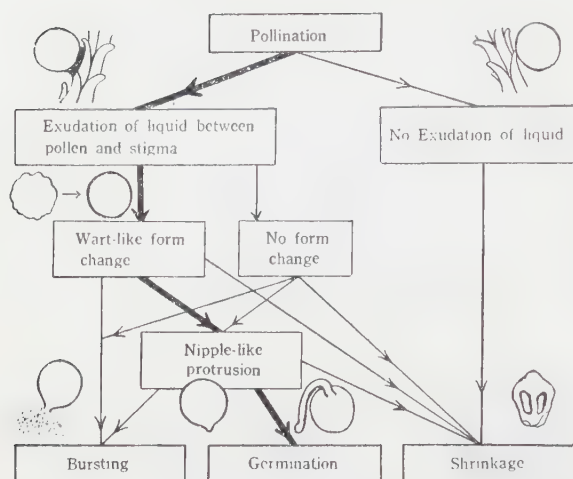


Fig. 9. Schema of the germination process of grass pollen

Prof. Dr. Shun-ichiro Imamura for his constant aid and criticism in the planning and carrying out of this study. He also wishes to express his sincere gratitude and appreciation to Mr. Kazuo Kato, of the Institute of Botany, College of Science, Kyoto University, for his suggestions and encouragement. For some of the materials the author is indebted to the Laboratories of Genetics and Crop Science, Faculty of Agriculture, Kyoto University.

Summary

1. Viable pollen grains exude a liquid as soon as they adhere to the stigma, manifesting at the same time a form change by the formation of wart-like bulgings covering the surface. This apparent form change proceeds after a definite pattern, and the grains return to their original smooth shape in about thirty to sixty seconds from the beginning of the process. The phenomenon was recognized hitherto in 28 species belonging to 19 genera of Gramineae.

2. The liquid accumulates at the point of contact of the grain with a stigma hair, and fills out the space between them. The liquid can be always observed when germination occurs, whereas pollen grains which do not exude it are incapable of germination.

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The wart-like form change is a very conspicuous and readily observable phenomenon. By its frequency, we can easily estimate the quality of pollen in respect to germination immediately after pollination. However, in the case of maize one has to be careful, because the wart-like bulgings sometimes appear rather late, e. g. forty to fifty minutes after pollination. In such cases the progress of the form change is also sluggish.

The author wishes to acknowledge his great indebtedness to

Physiological Changes in the Germination Seeds during the Low Temperature Treatment I

On the Water Content, the Specific Gravity of the Tissue Powder
and the Concentration of Reducing Sugars of Horse Bean

by Masaki YAHIRO* and Takashi INOUE

八尋正樹・井上 高：低温処理の発芽種子の生理的变化 I. 水分含量粉末比重及び還元糖について

Received December, 4, 1954

Introduction

In some plants the flower initiation is accelerated by subjecting the seeds to a low temperature treatment during the first stages of germination. Many investigations were hitherto made as to the mechanism of the vernalization with few satisfactory results.

The authors carried out several experiments with horse beans to see the inner physiological changes taking place during and after the vernalization procedure. As the water content of the cell as well as of the tissue had been considered to have usually a close relationship with the reactions occurring in the plant, the variation in the amount of water during the course of vernalization was first investigated. The specific gravity of the tissue powder was also measured, because this had been introduced by Koketsu^{1, 2)} and regarded as a suitable index giving a reasonable standard at the quantitative investigation. Finally the authors studied on the changes in the amount of reducing sugars by low temperature treatment.

This investigation was carried out in the Botanical Laboratory Faculty of Agriculture, Kyushu University.

The authors are grateful for the kind suggestion and encouragement given them by Professor Hitoshi Kojima; and they are also indebted to Ass. Professor Yoshio Tashima of Kagoshima University for his valuable suggestions during the course of the experiment.

Material and Methods

As the material some of the usual garden varieties of horse bean were used, because they germinate at any time from the early spring to the beginning of summer. The material was divided into two groups by the colours of the seed coat:

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namely green (that means the imperfect ripeness) and light brown (the perfect ripeness).

The seeds were immersed in water for 24 hours so that they absorb enough water; after the disinfection by a 0.01% solution of corrosive sublimate, they were washed thoroughly with water and put on a disinfected, suitably wet gauze in a china container and placed in an incubator of 25°C. After about 40 hours the seeds sprouted to a length of 4 mm. to 5 mm. The material, selected from these seeds, were subjected to a temperature of 0°C or 7°C with the control, the durations of the treatment being 7-, 9-, 14-, and 21-days. When the treatment was finished, a part of the material was used for measuring the physiological features cited below, and another part of the material was planted on pots, which were placed in a greenhouse in order to protect them from the coldness of early spring.

To calculate the water content of the seed, the authors removed the seed coat and weighed the naked embryo only, because the water, absorbed in the seed coat as well as contained between the coat and the embryo, was thought to have not only no physiological significance in the present study, but also there was some possibility to make the confused or disturb the estimation.

The specific gravity of the tissue powder was determined by weighing a unit powder-capacity of the material—or an unit volume of powder specially measured after Koketsu's powder method.

Reducing sugars were measured by Bertrand's method, and the sugar content was denoted by its amount contained in an unit powder-capacity of the material.

In order to test whether the vernalization was successful or not, fifteen plants of each lot of material were potted, as mentioned above, and the flower initiation was examined on April 25 and May 12. Number of leaves up to the node which bears the first flower bud, was also counted—the smaller thus number, the more advanced is the vernalization.

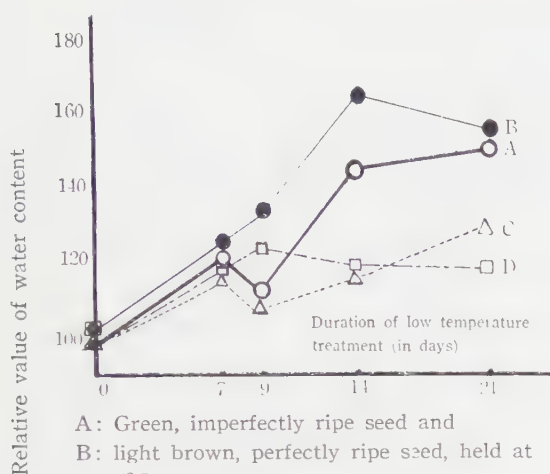
Experimental results and Discussion

1) Water content

The water content generally increased with the progress of the low temperature treatment as shown in Fig. 1. It is clear that the water is indispensable for various reactions which take place in the plant body and the increase in the water content generally indicates the increment in physiological activity of the plant.

The water content in both the perfectly ripe and the imperfectly ripe seeds after the treatment at 7°C was higher than that caused by the treatment at 0°C. From this fact and also from the value of specific gravity of the tissue powder (mentioned below), the lower temperature, 0°C, was seemingly too cold and caused the unfavorable changes in the plant tissues.

2) Specific gravity of the tissue powder



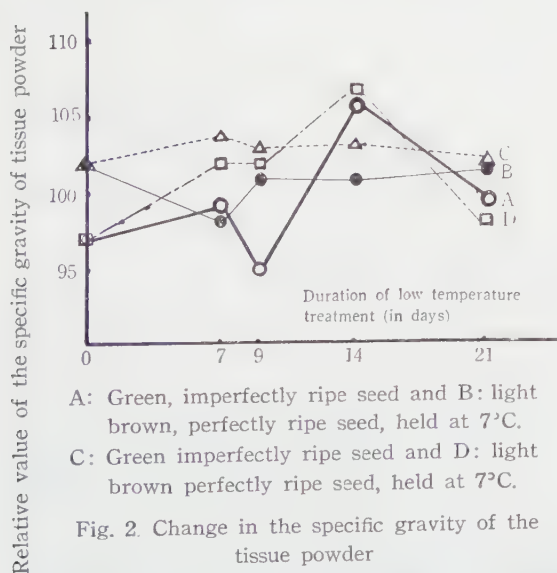
- A: Green, imperfectly ripe seed and
 B: light brown, perfectly ripe seed, held at 7°C.
 C: Green, imperfectly ripe seed and
 D: light brown, perfectly ripe seed, held at 0°C.

Fig. 1. Change in the water content

seems to have some relation with the vernalization, although it may be indirect collateral.

3) The amount of reducing sugars

As shown in table 3, there were found two peaks in the amount of reducing



- A: Green, imperfectly ripe seed and B: light brown, perfectly ripe seed, held at 7°C.
 C: Green imperfectly ripe seed and D: light brown perfectly ripe seed, held at 0°C.

Fig. 2. Change in the specific gravity of the tissue powder

The specific gravity of the tissue powder treated at 7°C was always greater than that treated 0°C, where the activity of the dissimilating reaction and the transformation and transportation of the substances were retarded by the low temperature.

Taguchi^{3,4)} reported that the specific gravity of the tissue powder of the mulberry tree increased in winter. It might be reasonable to say that this result shows the same tendency in mulberry tree as in horse bean as regards the effect of the low temperature. The specific gravity of the tissue powder

sugars of the material treated at low temperature, as to the length of the duration of the treatment. The material of 7-day treatment showed somewhat higher value and that of 14-day treatment highest. Generally speaking, this phenomenon can be seen in a similar was in the material treated at 0°C as well as in that treated at 7°C, almost regardless of the ripeness of the seed, perfect or imperfect. It is most likely that the increase in the reducing sugars to the higher activity of the hydrolyzing enzymes.

An unexpected extraordinarily shortness in the value of the reducing sugars content (and indeed this was the result of two parallel experiments) in the imperfectly ripe seed treated at 0°C for 14 days, could not be explained exactly.

The works in the winter wheat by Ovekin and others (1936) indicated that there was no relation between the amount of sugars and the vernalization; on the other hand Gregory and Purvis (1936), Gregory and De Ropp (1938), and Yamasaki (1943, 1944) put stress on the importance of sugars. The first of these authors stated that the growing embryo of the grain is able to synthesize hormones from the substratum containing glucose and inorganic salts. Gregory and De Ropp showed

- A: Green, imperfectly ripe seed and B: light brown, perfectly ripe seed, held at 7°C.
C: Green, imperfectly ripe seed and D: light brown, perfectly ripe seed, held at 0°C.

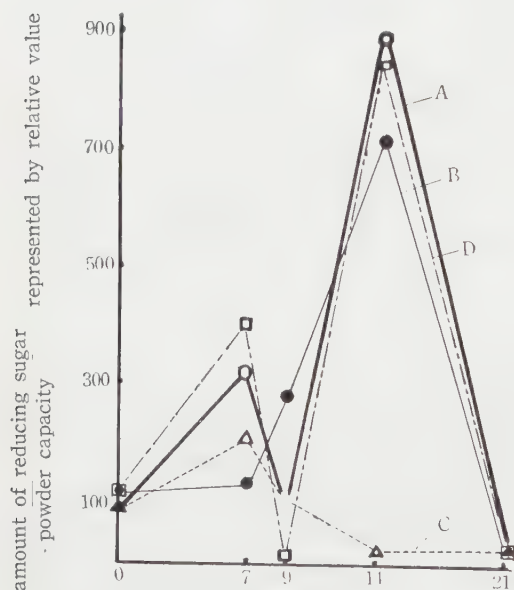


Fig. 3. Change in amount of reducing sugars

further that if sugar is excluded from the medium in which the excised embryos were to be grown, they remained unvernallized. Recently Tashima¹⁰⁾, Imamura and Tashima¹¹⁾ pointed out the importance of sugars to vernalize the embryo of radish plant in total darkness. On the other hand Kojima, Yahiro and Inoue¹²⁾ concluded that the supply of sugars is not strictly necessary for the vernalization of cotyledonless radish seedlings, although the supplying is somewhat favorable to vernalize them.

To the authors' regret, owing to the lack of untreated material (control), the material treated at low temperatures could not be compared with the control, in regard to the sugar content in the lapse of treatment.

4) Investigations on the first flower bud and flowering

The effect of vernalization increases with the progress of the low temperature treatment, as seen in Tables 3 and 4; and the temperature of 0°C is more effective than 7°C. Perfectly ripe and imperfectly ripe seeds which were treated at 7°C for 21 days showed 100% flowering within 47 days after planting, and on the other hand those which were not treated at a low temperature indicated only 20% flowering in the case of the imperfectly ripe seed, and 0% in the perfectly ripe seeds within 68 days after the planting, respectively.

The authors also ascertained in the field experiment that the number of leaves up to the first flower bud decreased with the progress of the low temperature treatment a sign of effective vernalization.

It is noticeable that the fall of flower buds prior to the flowering was often

observed on the plants grown up from the seedlings treated at low temperature during insufficient time length.

Table I. Condition of flowering on pots *

Duration of low temperature treatment (in days)	Days after the treatment		Days after the planting		A: Green, imperfectly ripe seed held at 7°C			B: Light brown, perfectly ripe seed held at 7°C			C: Green, imperfectly ripe seed held at 0°C			D: Light brown, perfectly ripe seed held at 0°C		
	April 25 May 12		April 25 May 12		**											
					a	b	c	a	b	c	a	b	c	a	b	c
0	52	68	52	68	40	20	10-11	40	20	10-11	0	0	—	0	0	—
7	52	68	45	61	73	73	9-10	40	27	10-11	—	—	—	0	30	11
9	52	68	43	59	80	73	9-10	47	53	10-11	57	57	9-10	200	40	10-11
14	52	68	38	54	87	80	8-9	61	67	9-10	57	57	8-9	20	50	10
21	52	68	31	47	87	100	7-8	61	67	9-10	100	100	8	—	—	—

*) Planted on March 3, 1950. The number of individuals planted: 15

**) Number of plants bearing flower buds (indicated by percent), a: Total number of plants, taken April 25. b: The same as the above, taken May 12. c: Number of leaves up to the node of the first flower bud.

Summary

1. With a variety of horse beans, the variation in the water content, the specific gravity of the tissue powder and the amount of reducing sugars caused by the low temperature treatment at 7°C and 0°C were studied.

2. The water content of the material treated at 7°C was much greater than that treated at 0°C.

3. On the contrary, the specific gravity of the tissue powder was smaller in the former case in the latter.

4. The amount of reducing sugars both in the material treated at 7°C and at 0°C showed the similar change and it increased with the progress of the treatment up to 14 days.

5. It was found that the treatment at 7°C was more effective than that at 0°C as regards to the vernalizing effect in the field experiment.

6. The effect of vernalization increased with the progress of the low temperature treatment and the period of 21 days was the most effective for vernalization in the present experiments.

7. The fall of flower buds prior to the sufficient flowering was sometimes observed on the plants grown from the seedlings which were treated at low temperature during insufficient time length.

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抄 録

N-carbamoyl 誘導体にしたアミノ酸のクロマトグラフィー

[Phillips D. M. P.: Chromatography of the N-Carbamoyl-amino acids
 Biochim. Biophys. acta **13**: 56 (1954)]

近年ペーパークロマトグラフィーは広く利用されているが、アミノ酸の面においても検出同定を容易にする為色々な展開剤、展開法が発表せられて来た。著者はアミノ酸を Carbamoyl 誘導体にする事により高い Rf 値を得、多種のアミノ酸の分離を容易にした。一方発色剤については Ninhydrine の利用と共に N-Carbomoyl アミノ酸, Citrulline, 尿素等には 4% p-Dimethylamino benzaldehyde による黄色反応を, Cysteine, Cystine には 2% Nitroprusside, 20% NaCO₃, 2M

NaCN 混液による赤色反応を又 Arginine にはアルカリ性 α-Naphthol 液と KBrO 液との混液による桃色反応を併用し検出を一層簡便にした。N-Carbamoyl 誘導体はアミノ酸溶液を多量の KCN と共に試験管中で 1~2 時間 100°C で熱する事により合成する、又此のものは展開後 Pyridine で溶出され比色定量する事も出来た。なお展開は二次元法を用いたが最良の結果を得た展開剤はブタノール、水、酢酸 (5:1:4) と水飽和 Phenol の場合であつた。(山本 茂)

花粉の生理学的研究 VIII

花粉管の伸長と核の行動について

岩 波 洋 造*

Yôzô IWANAMI: Physiological Researches of Pollen VIII
Growth of the Pollen Tube and Behavior of the Nucleus

1954 年 11 月 15 日受付

ま え が き

柱頭についた花粉の内容物(核その他)は花粉管によつて胚珠に運ばれる。花粉の核は発芽前に既に分れているものと、その後に分れるものがあるが、とも角前後2回の分裂によつて、2個の精核と1個の花粉管核(栄養核)を生ずる。両者共花粉管の伸長と共に子房に向つて進行するがこの中2つの精核は花粉管が胚珠の珠孔に達して破れた時、それぞれ卵核、極核と合して種子の元の細胞を作る。ところで花粉管核は如何なる働きをしているのであろうか? 従来漠然とその名の如く花粉管の伸長作用に關与していると考えられて来たようである。その理由は、花粉管核が花粉管の伸長時常に生殖核又は精核に先行して管の先端に位置しているとみられたこと、授精時になつて管の伸長が止まる頃には消失してしまうこと、それ以外の花粉管核の特殊な行動が見出されないことなどによるものであろう。しかしながら一方 Trankowsky¹⁾, O'Mara²⁾らは、花粉管核が時に花粉管中で精核の後に位置していることを見出し、Bishop³⁾ 筆者もこれを認めている。又 Brink⁴⁾ は培養基上で花粉管が分枝状伸長を行なうことを見ているし、筆者^{5, 6)}も又、人工的に容易にこれが作り出されることを報告している(この場合核をもたない花粉管が生ずると考えられる)。これらの観察の結果は次第に花粉管核の働きに対して疑問を深めつゝあつた。1953年 Bishop & Mc Gowan³⁾ は Colchicine で処理した *Tradescantia puldosa* の植物体から得た花粉が1核であることを見て、これを培養した結果、正常に花粉管が伸長することを知つた。更に管中に移

つたこの核が、最後まで分裂し得なかつたことに注目して、花粉管核は花粉管の伸長に不可欠なものでないこと、及び核分裂に關係しているものではないか? との考えに達している。筆者もこの問題に關しての2, 3の実験結果を得ているので、こゝにこれらを報告する次第である。

実 験

I: *Oenothera* の花粉が分枝状伸長を行うことが知られている外、花粉粒内が1核のまゝであることを知つたので、核がどのように花粉管中に移行するかを観察した。

方法は Sucrose 10%, Agar 1.5%, pH 6.5 の S. A. P. (蔗糖寒天板) 上に *Oenothera odorata* Jacq. の花粉を撒布し、一定時間毎に 27°C の湿室から出してスライドグラスにとり、5分間 Carnoy 液で固定した。これをスライドグラス上で Heulgen 反応の系列に移し、各々における核の位置を調べた。

図 1 は 1 時間後の花粉管の伸長状態と核の位置を示している。即ち 2~4 本の花粉管の中の 1 本にだけ核が存在し、他の花粉管は核をもっていないことがわかる。又必ずしも最長花粉管に核があるとは限らないし、一時間後にも核は 1 核のまゝで分裂していない。

II: *Impatiens Balsamina* L. の花粉は葯から放出される時、既に明らかに花粉管核と精核とに分れた 2 核をもっている。この花粉は高い濃度の Sucrose 培養基で培養すると 2~4 本の花粉管を生ずることが知られている⁸⁾。この場合の核の移動を観察した。

方法は Sucrose 24%, Agar 1.5%, pH 6.5 の S. A. P. 上に *Impatiens* の花粉を撒布し、30

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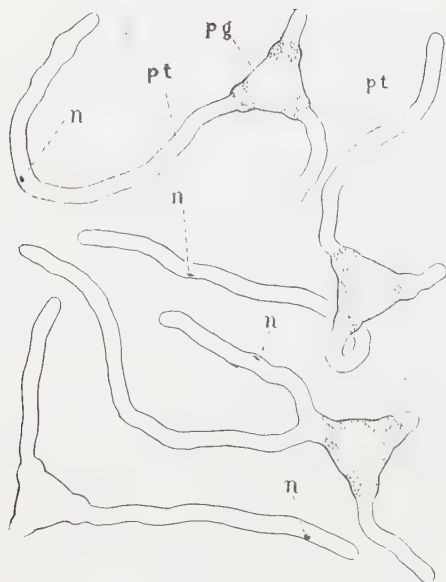


図 1 *Oenothera odorata* Jacq. の花粉管の伸長と核の位置 (1 時間後)
pg……花粉粒; pt……花粉管; n……核

分後これを前記同様 Heulgen 反応によつて核の位置を調べた。この場合も何れか 1 本の花粉管に核が移動し、稀には生殖核と花粉管核とが分れて別々の管に入っているものも見られた。即ち図 2 の如くである。

III: *Impatiens Balsamina* L. の花粉は前述の通り 2 核で管中に移り、そこで一方が分裂して 3 核となるが、花粉管核は常に生殖核に先行して花粉管中を進む。この *Impatiens* の花粉管が正常に伸長する時の、先端——花粉管核——生殖核の位置の変化を調査した。

方法は Sucrose 5%, Agar 1.5% pH 6.5 の S. A. P. に花粉を線状 (数層) に撒布し、それと直角に伸びてくる花粉管 20 個体について、Carnoy 液で固定、Aceto-carmin 液で染色後 Micrometer でその距離を測定、一定時間毎の平均値をとつてグラフに示した。

結果は図 3 に示された通りである。即ち花粉が発芽してから、管が 120~160 μ (花粉の長径の約 7 倍) になるまで核は管中に移行しない。やがて營養核が移り始めるが、生殖核はその後可成りの距離を置いて後から管中に移る。両者は共に急速に先端部に向つて進み、両者間の距離も又いじりしく短縮される。30~40 分後の花粉管の先端

部をみると、花粉管核と生殖核とは殆んど相接する如くにみられ、その後離れるが、しばらくして精核の分裂が開始される。図 4 がその状態を示している。

VI: Bishop³⁾ らの実験、及び実験 I の結果により、核分裂が行われなくても花粉管の伸長は進められることがわかつたので、逆に花粉管の伸長を停止させても核分裂は行われるかどうかをみる為、花粉管が伸長し得ないようにして花粉を培養し、核の行動を観察した。

方法は *Impatiens Balsamina* L. の花粉を、一旦 25% Sucrose の S. A. P. で 15 分間培養した後、5% Sucrose の S. A. P. に移して培養を続けた (これは通常 *Impatiens* の花粉が異なる濃度の培養基に移されてもよく適応して生長を続けること、及び高濃度の培養基では管膜が厚く形成される為以後の花粉管の伸長が起り得ないこと^{7,8)} を利用したものである)。

結果は花粉粒内に封じ込まれた 2 つの核は時間と共に図 4. の如くに変化した。これらの変化は花粉が発芽して管中に移つた時の核の変化と殆んど時間的に一致している (図 5) ことがわかる。

V: 花粉管核 (營養核) が花粉管の營養に関係しているかどうかについて調べるため *Impatiens*

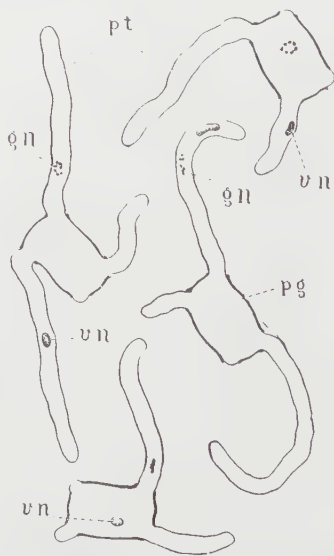


図 2. 花粉管を多く出した時の核の移動 (*Impatiens Balsamina* L.)
vn……花粉管核; gn……生殖核

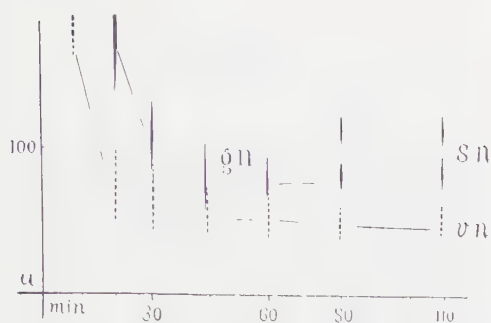


図 3. 花粉管の先端から、栄養核、精核までの距離の変化
sn……精核；vn……花粉管核；gn……生殖核

の花粉を生理的飢餓の状態の下において核の行動を見た。方法は先述⁸⁾の通り花粉粒内の澱粉粒を使い果させた花粉を寒天のみの培養基上で培養を続け、一定時間毎にこれをスライドグラス上にとつて Heulgen 反応で調査した。その結果は花

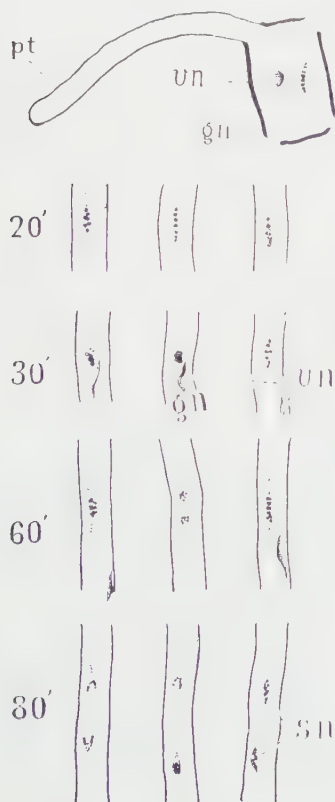


図 4. 花粉管の伸長にともなう核の変化
sn……精核；vn……花粉管核；gn……生殖核；pt……花粉管

粉管の伸長は停止したが花粉管核（栄養核）が消失するようなことはなく、行動は変るところがみられなかつた(図略)。

考 察

以上花粉管の伸長と核の行動についての観察の結果であるが、これらについて簡単に考察を加えると、Bishop らの研究と共に、以下に記す諸事実によつて、花粉管核（栄養核）は少くとも直接には花粉管の伸長とは関係がないと考えられる。

1. *Oenothera* の花粉は花粉管を数多く出すが、その中の一本だけが核をもつのみで他の花粉管は核をもっていない。



図 5. 花粉管の伸長を停止させた時の核の変化
vn……花粉管核；sn……精核；gn……生殖核

2. 通常一本の花粉管を出す *Impatiens* の花粉について人工的に 2~4 本の花粉管を出させた場合も同様なことがみられる。

3. 管の伸長時において、花粉管核は最後まで先端には達しないし、時にはこれが生殖核の後に存在することもある（最先端にあるものは帽体であつて核ではない）。

4. 花粉管の伸長は最先端における新たな管膜の形成（加附伸長）である⁹⁾。

5. 人工的に花粉管の伸長を停止させても核の行動には変化がない。

6. 生理的飢餓の状態においても花粉管核は消

失しない。

7. 核を含めないで原形質のみをとり出して培養した時膜を周囲に形成する⁹⁾。

なお花粉管核の存在意義については Colchicine で処理されてはいるが花粉管核をもたない核が分裂を行わなかつた Bishop らの実験、及び生殖核が分裂に先立つて花粉管核と接近することなどから、筆者は今の所花粉管核は生殖核の分裂に何等かの役割りをしていると考えている。たゞ両核の接近の前後における核の変化（大きさ、核酸など）について種々調査したが、明らかな違いがまだ認められていないことを附言しておく。

Resumo

Mi havis kelke da observadoj por klarigi funkcion de vegeta nukleo en la daŭro de la kreskado de polentubo sur la kulturaj medioj, precipe pri la agado de la nukleo.

Ĝiaj rezultoj estas subaj:—

1. En polenoj de *Oenothera* aŭ *Impatiens*, ne ĉiuj polentuboj enhavas nukleojn: t.e. iu polentubo en kiu enhavas nenion da nukleo.

2. Polenoj de *Oenothera* povas plene kreskigi polentubon, antaŭ ol la vegetaj nukleoj ankoraŭ disiras, se eĉ je unu nukleo.

3. La distancoj inter vegeta nukleo kaj genera nukleo kaj la ekstrema fino de la tubo estas ne konstanta. Ekzemple: genera nukleo alproksimiĝas provizore al vegeta nukleo antaŭ ol ĝia dividiĝo.

4. Polenoj transmititaj de 25% sukera solvaĵo al 5% solvaĵo ne ĝermas polenan tubon, tamen, interne de la poleno ĝia nukleo faras konduton same kiel en la polena tubo.

5. Laŭ nia ordinara kono, oni komprenas ke vegeta nukleo havas iun funkcion por kreskado de la polentubo, sed mi tamen pensas ke ĝi havas rilaton al la dividado de la genera nukleo.

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蘚類の蒴歯の発生学的研究 I

タマゴケの蒴歯の発生について

斉藤真太郎*・下瀬 敏**

Shintaro SAITO and Satoshi SHIMOZE: Studies on the Development of the Peristome in *Musci* I. On the Peristome in *Bartramia crispata* Schimp.

1954 年 12 月 20 日受付

蘚類のさく歯の構造及び発生状態は蘚類相互間の系統関係を示すものとして重要視されている。この方面から Cavers の分類による 4 類のうち、Eubryales は Philibert によれば Encalyptaceae を除いて Diplolepideae と Haplolepideae とに分けられている。Diplolepideae に属するものでは *Funaria hygrometrica* (Goebel, 1887, Campbell, 1905), *Mnium hornum* (Strasburger, 1905), *Mnium microphyllum* (Saito & Nishita, 1954) の研究があり、Haplolepideae に属するものでは *Ceratodon purpureus* の精密な研究がある。これらのさく歯はさく蓋部の同心円層から発生する内外 2 層の細胞層がさく歯の発生に直接関与し、その外さく歯層の細胞数は種により 16 であるが、内さく歯層の細胞数は種類により不定である。たとえば *Funaria hygrometrica* (ヒョウタンゴケ), *Mnium hornum* (オオヤマチヨウチンゴケ) では 32, *Mnium microphyllum* (コバノチヨウチンゴケ) では 64 に近い数, *Ceratodon purpureus* (ヤネノウエノアカゴケ) では 24 (内さく歯層細胞に相当する細胞数) である。すなわち外さく歯細胞層の 16 列の細胞から外さく歯層が、内さく歯細胞層の 24~64 列の細胞から内さく歯層が発育する。そしてこの内外 2 層の細胞の外側膜上に肥厚が起る。ヤネノウエノアカゴケではこの 2 層の細胞間の膜上に起る。Diplolepideae, Haplolepideae を問わずこれらの内外 2 層の細胞列数の比はさく歯発生の研究に重要な観察点のひとつである。斉藤、西田のコバノチヨウチンゴケの研究では内外

両さく歯の発生に直接関係する細胞層は外さく歯細胞層に外接する 32 細胞からなる層を加えて 3 層とした。

Bartramia crispata (タマゴケ) も Diplolepideae に属するがコバノチヨウチンゴケに比し胞子体、内さく歯等の形態上の特徴から、この発生の研究にも重要な課題と考えられるので次のような観察を試みた。

材 料 と 方 法

タマゴケの胞子体は松江地方では 10 月初旬頃に肉眼でわずかに認められるようになり、その後次第に成長して 1 月下旬頃その長径 16~18 mm に達し、2 月初、中旬頃から蒴がふくらみ始めさく蓋部が明らかになる。3 月初旬頃になると急速度に成長して 3 月下旬~4 月上旬頃にさく蓋部が赤褐色に変わり、完熟する。この間毎週 1 回、必要に応じて 2 回の採集を行い観察材料とした。

胞子体の固定には Bouin's solution を使用し、固定後数日経てからさく蓋部を破損することなく硬いせん帽を除去することができた。切片は 10 μ に切り、染色には Delafield's Haematoxylin を用いた。

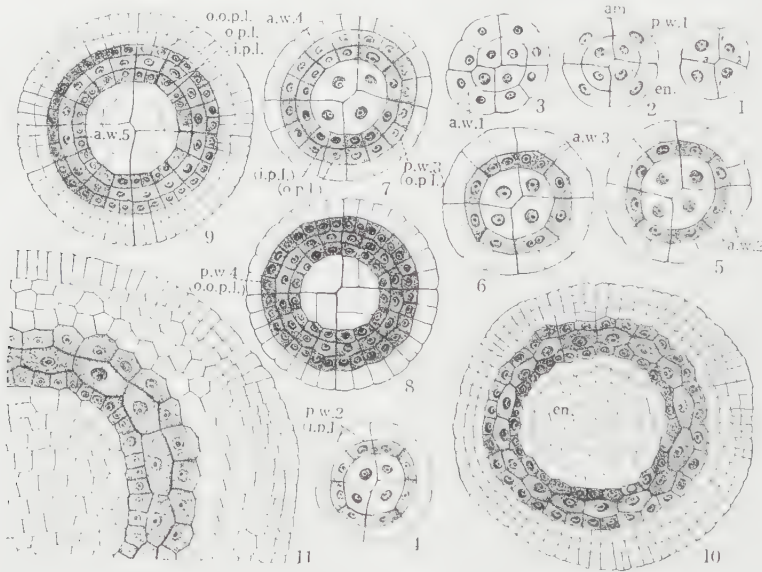
さく歯層の発育: タマゴケの胞子体もその発生初期 (10 月初旬) の横断面を見ると anticlinal walls によつて四分円に分れている (Fig. 1)。これがさく歯発生上の分裂の基礎であつて、この四分円の各細胞は最初の periclinal walls によつて amphithecium (原子嚢外層) と endothecium (原子のう内層) の内外 2 層に区分される (Fig. 2. am, en.)。この原子のう外層 (am) が最初の anticlinal walls (a. w. 1) によつて 8 細胞 (Fig.

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3) に分裂し、ついで第 2 の periclinal walls (p. w. 2) が生じて内外 2 層に分化する (Fig. 4)。この内層が anticlinal walls (a. w. 3) によつて分裂を重ねて将来の内さく歯層に内接する細胞層 (内さく歯細胞層) となるが、さく蓋部においてはこの層に起因する periclinal walls による分裂はこれ以上に起らない (Figs. 6~10)。他方外層は第 2 の anticlinal walls (a. w. 2) によつて 16 細胞に分かれ (Fig. 5), 続いてその内層の 8 細胞も分裂が進み (Fig. 6, a. w. 3), これと前後して第 3 の periclinal walls (p. w. 3) が生じて更に内外 2 層に分かれ、元の amphithecium layer は 3 層になる (Fig. 7)。この 3 層の中央の層が 16 細胞列からなる外さく歯細胞層となり、将来

この外側膜 (p. w. 3) に肥厚がおこる。外さく歯細胞層に外接する 16 細胞層は第 4 の anticlinal walls (a. w. 4) によつて 32 細胞層となり、これに第 4 の periclinal walls (p. w. 4) が生じて 32 細胞列からなるところの外さく歯外接細胞層ができあがる (Fig. 8, p. w. 4)。この層の細胞列は外さく歯の先端部に接する部位では外さく歯細胞層に等しく 16 細胞列になつている。タマゴケでは外さく歯細胞層が第 3 の periclinal walls (p. w. 3) によつてできあがる前後頃から内さく歯細胞層の 8 細胞に anticlinal walls ができて 16 細胞となり (Fig. 8), その後の胞子体の肥大成長に伴なつておおむね細胞の大きさが不等となり、外さく歯細胞に対応して細胞にずれを生じつ



Figs. 1~11. Cross sections through the opercular region of young capsules showing successive stages of development of peristomial layers stippled, $\times 300$: 1. Division of segments into quadrant; (1~3, developmental sequence) 2. Showing amphithecium and endothecium; 3. 8-celled stage of an amphithecium layer divided by anticlinal walls; 4. Showing an inner peristomial layer divided by periclinal walls; 5. 16-celled stage of an outer amphithecium layer divided by anticlinal walls; 6. Successive divisions of segments into 16-celled stage in an inner peristomial layer by anticlinal walls; 7. Stage showing an outer peristomial walls; 8. 16-celled inner and outer peristomial layers, accompanying circumscribed 32-celled layer, endothecium now under division; 9. Inner peristomial layer under division by anticlinal walls; 10. Both amphithecium and endothecial cells are much increased; 11. Completion of cell divisions in the region of opercle.

am. amphithecium, a. w. anticlinal walls (1~5, developmental sequence), en. endothecium, i. p. l. inner peristomial cell layer, o. o. p. l. circumscribed cell layer of outer peristomial cell layer, o. p. l. outer peristomial cell layer, p. w. periclinal walls (1~4, developmental sequence).

つ 32-celled stage を経て 48 に近い細胞数となる (Figs. 9, 10, 11)。胞子体の長さが 16~18mm に達した 1 月中、下旬頃さく蓋部の連続横断面で内さく歯の輪状に配列する各層の細胞数をさく蓋部の先端から順々に観察すると 16, 32, 38, 42, 47, 48, 49, 51, 52 等の変数の数が得られ、48-celled stage における外さく歯細胞層に内接する内さく歯細胞層は前者の 1 に対し 2 (又は 1~3) の比較的小さい細胞とこれをはさむ大形の細胞 (この細胞は 2 外さく歯細胞が接する部分に位置して多くはこの 2 外さく歯細胞にまたがっている) とが並んでいる (Fig. 11)。この大形細胞はさく蓋部の肥大成長につれて大きくなり、これにはさまれる小形細胞との大きさの差が著しくなる (Figs. 11, 14, 15)。前述のさく蓋部先端から順次数えた最先端の横断面に見た 16 細胞はさく蓋部の肥厚開始後の観察によっても、そして完成された内さく歯が外さく歯に比べてコバノチョウチンゴケのそれに見るよりもはるかに短いことから (Fig. 19)、内さく歯細胞と認めることはできないことがわかる。そして 32 の細胞数を示す部分は内さく歯の基膜部 (間毛に当る細胞を含む) に配列する細胞数の変数によるものである。

以上のような分裂によつて作りだされた内さく歯細胞層 (32~48 細胞列)、外さく歯細胞層 (16 細胞列)、外さく歯外接細胞層 (32 細胞列) の 3 層の細胞が内外両さく歯の形成に直接関与して特徴あるさく歯の形態をつくりあげる。内さく歯層は初期発生理、厳密な分裂順序を示さないで 32 細胞段階後 48 に近い細胞数に達して分裂を終る頃には外さく歯外接層の外側部も細胞分裂を重ねて 3~5 層の細胞層からなるところのさく蓋に発達してくる (Fig. 11)。この部分の分裂は外さく歯細胞層や外さく歯外接細胞層の分化のような規則正しさを一様に求めることはできないにしても anticlinal walls と aelical walls とが交互に現われることによつて分裂が決められるものと考えられる (Figs. 10, 11)。

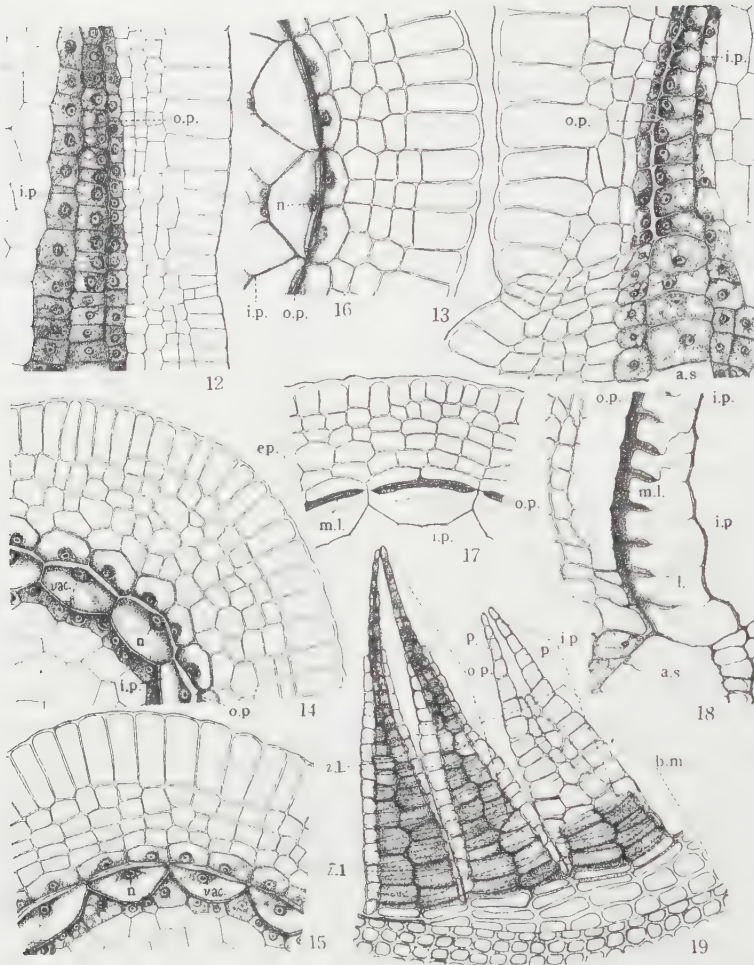
原子のう内層 (Fig. 2, en.) の分化も四分円が基盤となつて内さく歯細胞層に内接する内層全体において細胞分裂が起り次第にその数を増加していくがさく歯層の分裂に見られるような規律正しい分裂ではない (Figs. 8, 9, 10, 11)。さく歯の基部では外さく歯細胞層は anticlinal walls によつ

て 2 分され 32 細胞となり、更に胞子室に近い部分では 2 分されて 64 の細胞を数えることができる。

さく歯膜の肥厚: さく歯の形成が行われている細胞列は内さく歯細胞層 32~48 (i. p. 1.), 外さく歯層 16 (o. p. 1) 及び外さく歯外接層 32 (o. o. p. 1.) の 3 層の細胞で、特に内さく歯層と外さく歯層の細胞の 48 (32+16)~64 (48+16) 列は各 6~8 列ずつの 8 群に自然に分れる。すなわち外方のさく歯層 2 歯 (細胞 2) は内方さく歯層の細胞 4~6 に対応し、さく歯はその内さく歯層と外さく歯層の細胞のそれぞれ外側の periclinal walls の上から起るもので、内さく歯層と外さく歯層とではその肥厚の程度が著しく異なる。

肥厚の機構はコバノチョウチンゴケに類似している。さく歯形成の爲の分裂を終つた 1 月下旬頃、胞子体のさく蓋部の横断面や縦断面を見ると、この 3 層の細胞は細胞質に富み割合に大きくなつた核が中央部に認められて他の細胞層との区別が容易となる (Figs. 11, 12)。この頃から胞子体がふくらみ始めて、さく蓋部が見分けられるようになる。胞子体のふくらみが増すにつれて 3 層の細胞内にも変動が起り、細胞質が不均一となり、さく歯層に片寄つてきて空胞が生じ (vac.), 外さく歯層の基部の膜壁から他の細胞膜に比べてやや肥厚してくる (Figs. 12, 13, 14)。ついで外さく歯細胞層 (Fig. 9, o. p. 1.) と同外接細胞層の細胞質と核とは外さく歯層 (Figs. 12, 14, o. p.) の方に、内さく歯細胞層 (Fig. 9, i. p. 1.) の細胞質と核とは内さく歯層 (Figs. 12, 13, 14, o. p.) に向つて偏在しているのが認められ、活発な肥厚作用が営まれてきたものと思われる (Figs. 13, 15, 16)。特に外さく歯層の肥厚は顕著である。さく蓋基部の縦断面では肥厚の起らない胞子のうに近い部分が同一層上にありながら細胞質や核の偏在が認められない (Fig. 13)。肥厚が進むに従つて細胞質は膜壁に沈着したかの観を呈して次第に少くなり空胞が拡大する。完成したさく歯が認められるようになると核も消失してしまう (Figs. 17, 18)。

このように細胞内容の移行や消失が肥厚の開始や終了を物語るものとすれば、最も早く肥厚を始めるのは外さく歯細胞層の基部であつて先端部に



Figs. 12~19. Cross and radial sections through the amphithecial tissues of half-ripe capsules stippled $\times 300$, and 2 outer teeth and 3 inner teeth of ripe capsule stippled, $\times 200$; 12~13. Early stages of thickening of the outer teeth; 14~15. Showing maldistribution of cytoplasm and nuclei; 16. Early stage of disappearance of cytoplasm and nuclei, and destruction of the parenchyma of the inner layers of the inner peristomes; 17~18. Basal portions of the inner and outer teeth, showing the peristomial thickenings in their final stage of development; 19. Fully developed teeth (2 outer teeth and 3 inner teeth), seen from outside.

a. s. air space; b. m. basilar membrane; ep. amphithecial cell layer; i. p. inner peristome; o. p. outer peristome; l. lamella; m. l. middle lamella; n. nucleus; p. processus; vac. vacuole; z. l. zigzag longitudinal line.

うつるにつれて遅くなってくる。内さく歯外層の細胞層がこれに次ぎ、外さく歯細胞層外層細胞の核が多くの場合最も遅れて退化する (Fig. 16)。外さく歯膜と内さく歯膜とに連続している細胞膜にも外さく歯に連なる付け根の部分に肥厚が及んでいるので、これが薄板にあたり、縦断面でこの

ぎりの歯のように見える (Fig. 18, 1.)。外さく歯の先端部に移るに従って薄板はだんだん狭くなっている。これを付ける外さく歯自体の先端部は一種の弧を描いている。内外両さく歯壁を連結している薄膜は胞子体の完熟まで残存する (Fig. 18)。

なお外さく歯の外側面にはその外接細胞の膜と

連結している膜がわずかに肥厚して残存した低い突起として認められる。横断面では外さく歯外側中央部に anticlinal walls の一部がわずかに肥厚した突起として残る (Fig. 17)。

これを外さく歯外側から見るとその基部から先端に向つて中央を走る zigzag longitudinal line となつてゐるが外さく歯の先端には及んでいない。このことはさく蓋先端部の横断面で外さく歯外接細胞が 16 となつてゐることと一致する。完成した外さく歯の横断面や縦断面を観察すると細胞中膜の内側と外側の肥厚状態がよく見られる。その内側 (Figs. 17, 18) は外さく歯細胞層の細胞によつて肥厚した部分であり、その外側 (Figs. 17, 18) は外さく歯外接細胞層の細胞によつて肥厚した部分であることが察され、しかも両者の厚さの差は全く認められない。内側の肥厚部は赤褐色を、外側には赤褐色を呈している。そしてこの外さく歯の外側肥厚部表面と内さく歯内面にも見られる微小乳頭状突起が密生している (Fig. 18)。内さく歯に内接する細胞が崩壊すると共に外さく歯外接する細胞膜にも破壊が起り、両さく歯がさく歯に蓋から独立するが外さく歯の基部 2~3 層の外さく歯外接細胞は破壊しないで残り、外さく歯をさく壁に連結している (Fig. 18)。この部分の肥厚壁中には微小乳頭状突起が認められず、色も内側の肥厚壁と同じく赤褐色である。外さく歯の厚さが 7~8 μ 程あるのに対し、内さく歯の厚さは 1.5 μ 位の薄い膜であつてその中膜 (m. l.) を境として両側の肥厚に赤褐色にもその色は認められない。内さく歯の長さは外さく歯の 2/3 余りで基礎膜の高さは全長の 1/2 を占め間毛としての発達部はないが間毛部に当る細胞列 (1~3 列) が基礎膜 (b. m.) に含まれ、これをささむ 2 本の歯突起 (p.) が外さく歯 1 本に対応して 16 groups をつくつてゐる (Fig. 19)。

一方外さく歯外接細胞層の外側は 3~5 層の細胞からできて (Fig. 17, ep.), 次第に強固となり、特にその表皮はさく歯層の肥厚に前後して肥厚してくる。3 月中、下旬頃からさく蓋部が赤褐色になり、さく歯は全く完成されて内さく歯内側の柔細胞の崩壊も始まつてゐる (Figs. 17, 18)。そして外さく歯とその外接層の間も破壊して Fig. 19

の状態になるのは孢子体成育の最終期である。このようにしてさく歯層上の肥厚も完成し、さく歯細胞が乾燥すると、その膜の薄い部分はしなびて見えなくなり、歯が分離する。同時に蓋板の基部もさく歯部の發育につれて内側組織が崩壊し環部 (Mnium に見るような ring はない) に当る外側基部に離層が生じてさく蓋が落ちて湿気に鋭敏な運動をする。

考 察

Bryales では若い孢子体の發育の規則正しい進行は全体としてもつ特徴であることは Kuntzen (1912) が力説していることで、ヤネノウエノアカゴケ 1)、コバノチョウチンゴケ 2)、等の孢子体の横断面にも明らかに現われてくるようにタマゴケでも観察の結果が一致した。

外さく歯細胞層の 16、外さく歯外接細胞層の 32 の細胞数はヤネノウエノアカゴケ、コバノチョウチンゴケと全く同数である。

内さく歯細胞層の細胞数はヒョウタンゴケとオオヤマチョウチンゴケでは 32、ヤネノウエノアカゴケでは 8-celled stage から特殊な分裂によつて細胞数 24 となり、コバノチョウチンゴケでは 8, 16, 32 までの細細分裂は規則正しいが 32-celled stage から不規則となり、32~64 に近い細胞数に達するのとは異なり、タマゴケでは 48 に近い細胞数を認めることができた。なおコバノチョウチンゴケの 64-celled stage に大小不等の細胞からできてくる状態を見るとヤネノウエノアカゴケのように不均等な分裂によつて生ずるのとは異なり、その成育の過程においてずれを生じ、細胞の形状大小に変化をきたしたものと観察の結果考えられるのと同じ結果をタマゴケの 32-celled stage を経て 48-celled stage になる間に形状の変化とそれぞれのずれを認めることができた。

タマゴケの外さく歯の形態はコバノチョウチンゴケに類似しているのに対し、内さく歯は著しく相違する。

おわりに臨み本研究を進めるに当つて懇篤なる御指導と校閲を賜つた大分大学学芸学部教授野口彰博士に深甚なる謝意を表する。

Summary

1. The initial quadrant, showing 4-celled stage in cross section, is divided by the first periclinal walls into the amphithecium and the endothecium. The original amphithecium is divided by the second periclinal walls into inner and outer layers.

2. The inner peristomial layer arises from the inner amphithecial layer, and comes to be composed of 32~48 longitudinal rows of cells by the formation of anticlinal walls.

3. By the formation of periclinal walls the amphithecium is divided into outer and inner cell layers, of which the inner layer comes to have sixteen cells in cross section by forming anticlinal walls. These sixteen cells undergo no further division.

4. The ridges of thickening, representing the teeth, are laid upon each of the outer periclinal walls of inner and outer peristomial cells, respectively.

5. Before the deposition of the peristomial thickenings, the nucleus in the cells relating the formation of teeth is enlarged. And then the protoplasm of both the inner and outer peristomial cells moves towards their outer sides respectively, while these of circumscribed cells towards their inner sides. Accompanying with the thickening the cytoplasm and nuclei gradually shrivel away and disappear.

6. The thickening of the inner teeth begins at the same time with that of the outer teeth. However, the thickening in the upper portions of the outer teeth happens later than that in their basal portions.

7. After the formation, the processes of the inner peristome longitudinally splits along the keel line.

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The Effects of Certain Metabolic Inhibitors on the Dark Reaction during Photoperiodic Treatment

by Shidai NAKAYAMA*

中山至大：暗期反応に及ぼす代謝阻害剤の影響

Received January 11, 1955

There exist a great deal of reports on photoperiodism, but little work has been done in order to elucidate the enzyme systems involved in the dark reaction during photoperiodic treatment by using metabolic inhibitors¹⁾. This paper reports a preliminary study on this problem.

The seedlings of *Pharbitis Nil* (a short day plant) cultured under the continuous light were used as the material. The fully expanded cotyledons of the seedlings which had received 8-hour's light period were immersed in *M*/15 phosphate buffer of pH 5.0 (pH 4.7 in the case of malonic acid) containing a given concentration of each inhibitor during 16-hours dark. After such treatment was repeated three times, the seedlings were brought back under the continuous light and held for one week. Flower initiation was then observed.

Malonic acid, even in relatively high concentrations ($2 \times 10^{-3}M$, $1 \times 10^{-2}M$, etc.) did not inhibit flower induction. Hence, it seems that the dark reaction could proceed without the TCA cycle. In other words, it appears that, as reported by Newcomb and Stumpf²⁾ and Ôta³⁾, the TCA cycle may not be involved in the cotyledons of *Pharbitis* seedlings. However, Liverman *et al.*⁴⁾ have found that some of the intermediate products of the TCA cycle were effective to the flower induction, when given to them.

The results obtained by using cyanide, fluoride, azide, and arsenate are summarized in Table I. As shown in it, flower induction was completely inhibited by cyanide and azide at certain concentrations. Therefore, the dark reaction appears to be closely linked with the heavy metal-containing enzymes (iron enzymes, copper enzymes, etc.). Inhibition by fluoride suggests the presence of a relationship between the dark reaction and the glycolytic phase of respiration.

That azide prevented the flower induction suggests that the dark reaction may be dependent upon the phosphorylation in glycolysis process. Little inhibition by arsenate seems to be due to phosphate buffer; accordingly, this point need further confirmation by experiments using other buffers.

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Table I. Inhibition of floral initiation

Treatment	Average number of flower bud per plant	Percentage* flowering
None (air)	3.4	100
phosphate buffer	2.8	100
5.0×10 ⁻⁵ M cyanide+phosphate buffer	1.3	83.3
1.0×10 ⁻⁴ M cyanide+phosphate buffer	0	0
2.5×10 ⁻⁴ M fluoride+phosphate buffer	1.9	100
5.0×10 ⁻⁴ M fluoride+phosphate buffer	0.8	66.7
None (air)	4.3	100
phosphate buffer	3.9	100
2.5×10 ⁻⁵ M azide+phosphate buffer	0	0
5.0×10 ⁻⁵ M azide+phosphate buffer	0	0
5.0×10 ⁻⁵ M arsenate+phosphate buffer	3.4	100
1.0×10 ⁻⁴ M arsenate+phosphate buffer	3.6	100

* Twelve seedlings were used for each treatment

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編集後記

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Some Marine Cyanophyceae of the Tokara Islands

by Isamu UMEZAKI*

梅崎 勇: トカラ群島の海産藍藻類

Received December 20, 1954

The present study of the marine Cyanophyceae deals with a collection found in materials of algae collected by Mr. Eiji Ogata in the Tokara Islands, which stretch in the range from 29° to 30° N. L. between the southern end of Kyushu and Okinawa. Mr. E. Ogata, one of the scientific research members of the Tokara Islands under the management of Mr. Yoshitaka Tsutsui, Director of the Osaka Municipal Museum of Natural History, made researches and collections of marine algae in the islands during the period from May to July of 1953, and after returning to his laboratory, he sent the writer a collection of marine Cyanophyceae from abundant materials of marine algae for identification.

Materials fixed in 5~6 per cent seawater-formaline were preserved in three glass bottles, one of which was collected at Takara-zima on May 27, and two at Nakano-shima on June 5. The former one is being preserved as Umezaki's Collection No. 1139 and the latter two as Umezaki's Collection No. 1140 and No. 1141 in Umezaki's herbarium respectively.

Though it was a small collection, the writer has identified marine Cyanophycean species of 21 in all, including one species new to science and two species new to Japan.

The writer wishes to tender his best thanks to Dr. Y. Yoneda for his kind direction during this work, and to Dr. F. Drouet of the Chicago Natural History Museum for reading the manuscript. Thanks are also due to Mr. E. Ogata of the Osaka City University, who kindly sent the writer his valuable specimens from the Tokara Islands.

Family Pleurocapsaceae

Genus *Myxohyella* Geitl., 1925

Myxohyella socialis (Setch. et Gardn.) Geitl., Cyan. in Rabenh., Kryptogamen-fl. **14**: 381, fig. 211 (1931); *Hyella socialis* Setch. et Gardn., New Pac. Coast Alg. 2, Univ. Calif. Publ. Bot. **6**: 443, pl. 36, fig. 5 (1918).

Filaments up to 75 μ in length; cells rectangular, 3~6 μ in diameter, terminal

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cells up to 17μ in length.

Hab. Nakano-shima (Jun. 5, 1953). In the filaments of *Heterosiphonia pulchra* (Okam.) Fkgb. Scarce. No. 1140 e(in Umez. herb.).

The terminal cells of the filaments are very long and large, attaining 17μ in length. However, according to the original diagnosis under the name of *Hyella socialis* by W. Setchell and N. Gardner, the terminal cell is described to be $7\sim9\mu$ in length.

Genus *Pleurocapsa* Thur., 1885

Pleurocapsa fuliginosa Hauck,; Meeresalg. in Rabenh., Kryptogamen-fl. 2: 515, fig. 231(1885).

Cells $6\sim26$ (~33) μ in diameter; cell membrane up to 3.5μ in thickness.

Hab. Takara-zima (May 27, 1953). On rocks, associated with *Hydrocoleum lyngbyaceum* Kuetz. and *Lyngbya aestuarii* (Mert.) Liebm. f. *spectabilis* (Thur.) Gom. Scarce. No. 1139 k(in Umez. herb.).

Family Dermocarpaceae

Genus *Dermocarpa* Crouan, 1859

1. Cells $7\sim16\mu$ in diameter.....*D. sphaerica*

1. Cells $14\sim24\mu$ in diameter.....*D. sphaeroidea*

Dermocarpa sphaerica Setch. et Gardn., in Gardn., New Pac. Coast Alg. 3, Univ. Calif. Publ. Bot. 6: 457, pl. 39, fig. 14 (1918).

Cells $7\sim16\mu$ in diameter, pale blue-green; endospores $2.5\sim3\mu$ in diameter.

Hab. Nakano-shima (Jun. 5, 1953). Growing on *Lyngbya* sp. Scarce. No. 1140 h(in Umez. herb.).

Dermocarpa sphaeroidea Setch. et. Gardn., in Gardn., New Pac. Coast Alg. 2, Univ. Calif. Publ. Bot. 6: 440, pl. 36, fig. 7 (1918).

Cells spherical or obovate, $14\sim24\mu$ in diameter; cell walls $1.5\sim2.5\mu$ in thickness; endospores formed by simultaneous divisions of the whole protoplast, $2\sim2.5\mu$ in diameter.

Hab. Nakano-shima (Jun. 5, 1953). On *Lyngbya confervoides* C. Ag. and *Heterosiphonia pulchra* (Okam.) Fkgb. Abundant. No. 1140 d(in Umez. herb.).

In 1918 N. Gardner established *Dermocarpa sphaeroidea*, based upon materials growing on *Porphyra perforata* f. *lanceolata* from Land End, San Francisco, California, collected on April 1917.

The present species of Nakano-shima is abundantly found epiphytic on *Lyngbya confervoides* C. Ag. and a red alga *Heterosiphonia pulchra* (Okam.) Fkgb. The cells grow solitary or forming more or less gregarious small clusters on host algae, and the cell walls measured $1.5\sim2.5\mu$ in thickness are somewhat thicker than those of the original specimens. In regard to this species from the San Juan Islands of the

San Juan Archipelago, Washington, C. Jao (1948) described that the cells attain sometimes 34μ in diameter and the endospores are $3.5\sim5\mu$ in diameter. However, in the original description given by Gardner, he did touch neither on a process of the division of sporangia nor on the dimension of endospores. In the specimen at hand, it is observed that endospores which are $2\sim2.5\mu$ in diameter are formed by simultaneous divisions of the whole protoplast of a sporangium. It is for the first time that the present species is added to the marine Cyanophycean flora of Japan.

Family Rivulariaceae

Genus *Calothrix* C. Ag. ex Born. & Flah., 1886

Calothrix pilosa Harvey ex Born. & Flah., Rev. Nost. Hétér. I, Ann. Sc. nat. Bot. VII, 3: 363 (1886).

Filaments up to 0.5 cm in length, $22\sim37\mu$ in diameter; sheaths up to 9μ in thickness; trichomes blue-green, $15\sim24\mu$ in diameter; heterocysts $3\sim36\mu$ in length; cells $3\sim10\mu$ in length.

Hab. Nakano-shima (Jun. 5, 1953). Growing on rocks. Abundant. No. 1141 (in Umez. herb.).

Family Nostocaceae

Genus *Anabaena* Bory ex Born. & Flah., 1886

Anabaena sp.

Filaments forming blue-green small strata or intricate associating with other Cyanophyceae; sheaths mostly diffluent, hyaline, thin; trichomes sometimes slightly tapering at the ends; cells barrel-shaped, quadrate or about 2 times shorter than the diameter, $5.5\sim6.2\mu$ in diameter, $3\sim7.6\mu$ in length; terminal cells rounded or slightly conical; heterocysts quadrate or somewhat longer than the diameter, $7\sim8.5\mu$ in diameter, $6.2\sim9.5\mu$ in length; spores unknown.

Hab. Takara-zima (May 27, 1953). Growing on rocks and associated with *Hydrocoleum cantharidosmum* (Mont.) Gom. and *Phormidium penicillatum* Gom. Abundant. No. 1139 h (in Umez. herb.).

The present material of *Anabaena* was abundantly found forming small colonies on rocks or mostly associating with other blue-green algae. The filaments have sometime hyaline mucous sheaths. During a stage when the filaments are young the trichomes are slightly attenuated at their ends and their terminal cells are somewhat smaller than those of the middle part. The dissepiments of the trichome cells are deeply constricted and conspicuously pellucid. Unfortunately, in despite of careful observations the writer could not found spores which are an important characteristic for specific determination, therefore no identification could be done. Though from the reason noted above the material remains as a question, in the dimension of the cells and heterocysts it agrees well with the description of *Anabaena variabilis*.

Genus *Nostoc* Vauch. ex Born. & Flah., 1888

Nostoc commune Vauch. ex Born. & Flah., Rev. Nost. Hétér. IV, Ann. Sc. nat. Bot. VII, 7: 203 (1888).

Thallus 3.5 cm in diameter, membranaceous, yellowish green; cells 5~6 μ in diameter, 4~6.2 μ in length; heterocysts ovoidal, 6.3~7.7 μ in diameter, 7~9 μ in length.

Hab. Takara-zima (May 27, 1953). On rocks. Single specimen. No. 1139 a(in Umez. herb.).

Only a single specimen was found on rocks' forming a membranaceous, somewhat gelatinous and yellowish green mass, being 3.5 cm in diameter. The sheaths are distinctly recognized and are very thick near the surface layer of the frond, but not visible in the inner layer. Each cell of the trichome sometimes contains dense granules. The intercalary heterocysts are usually single at each position instead of being serially two or more.

Family Oscillatoriaceae

Genus *Spirulina* Turpin ex Geitl., 1925

1. Trichomes 0.5~0.7 μ in diameter; dissepiments obscure *S. socialis*

1. Trichomes 6 μ in diameter; dissepiments distinct *S. attenuata*

Spirulina socialis Gardner, Myxophy. of Porto Rico and Virgin Isl., Sc. Surv. Porto Rico and Virgin Isl. 8: 272, pl. 1, fig. 1 (1932).

Trichomes 0.5~0.7 μ in diameter, pale blue-green; coils 1.2~1.5 μ in diameter, the distance between coils 1.2~1.8 μ .

Hab. Takara-zima (May 27, 1953). On *Hydrocoleum lyngbyaceum* Kuetz. Abundant. No. 1139 n(in Umez. herb.).

This species is a fairly minute *Spirulina* which is found epiphytic on the sheaths of filamentous blue-green algae. The regularly coiled trichomes are very long and nearly straight.

Spirulina attenuata Umezaki, Mar. Cyan. fr. Jap. 4, Journ. Jap. Bot. 27: 117, fig. 17 B(1952).

Trichomes 6 μ in diameter, light aeruginous; cells 2~3 μ in length.

Hab. Takara-zima (May 27, 1953). Among the filaments of *Lyngbya aestuarii* (Mert.) Liebm. f. *spectabilis* (Thur.) Gom. Scarce. No. 1139 m (in Umez. herb.).

Genus *Oscillatoria* Vauch. ex Gomont, 1892

Oscillatoria chalybea (Mert.) Gom. var. *genuina* Gom., Monogr. des Oscill., Ann. Sc. nat. Bot. VII, 16: 233 (1892).

Trichomes light blue-green, 12.3~14.5 μ in diameter; cells 3~6 μ in length.

Hab. Takara-zima (May 27, 1953). Among the filaments of *Hydrocoleum can-*

tharidosmum (Mont.) Gom. and others. Scarce. No. 1139 (in Umez. herb.).

Judging from the diagnosis of *Oscillatoria chalybea* this specimen seems to be probably identified with it, although the trichomes are a little thicker and the cells are a little shorter.

Genus *Phormidium* Kuetz. ex Gomont, 1892

1. Trichomes $4\sim5\mu$ in diameter; terminal cells sharply conical, without calyptra; cells $3.7\sim9.3\mu$ in length *P. Corium*

1. Trichomes 6μ in diameter; terminal cells flattened or rounded, with calyptra; cells $6\sim12\mu$ in length *P. penicillatum*

Phormidium Corium (Ag.) Gom., Monogr. des Oscill., Ann. Sc. nat. Bot. VII, 16: 172, pl. 5, figs. 1, 2(1892).

Filaments densely intricated; sheaths thin, sometimes diffluent; trichomes briefly or sometimes gradually tapering at the terminals, $4\sim5\mu$ in diameter, not or sometimes slightly constricted at the cross walls; cells $3.7\sim9.3\mu$ in length; terminal cells sharply conical, neither capitate nor calyptrate.

Hab. Nakano-shima (Jun. 5, 1953). Growing on rocks. Abundant. No. 1140 a(in Umez. herb.).

This species grows forming widely expanded membranaceous masses on rocks. According to the description of the species the diameter of trichomes is described to be $4\sim4.5\mu$. In the material at hand it is $4\sim5\mu$. There are trichomes to be distinguished into two types in Nakanoshima's specimen, concerning constriction at their cross walls; some trichomes are not constricted at their cross walls as those of the species, and other ones are constricted at their cross walls as those of var. *constrictum* Playfair. The material may be rather identified with var. *constrictum* in having a little thicker trichomes and in the characteristics that some trichomes have constrictions at their cross walls, but herein it is placed under the specific name *Phormidium Corium* (Ag.) Gom. for showing both mixed feature as pointed out above.

Phormidium penicillatum Gomont ex Geitl., Cyan. in Rabenh., Kryptogamen-fl. 14: 1016 (1932).

Filaments intricated; sheaths thin, mostly diffluent; trichomes 6μ in diameter; cells $6\sim12\mu$ in length; terminal cells with flattened, conical or rounded calyptras, sometimes capitate.

Hab. Takara-zima (May 27, 1953). On rocks, associated with *Hydrocoleum cantharidosmum* (Mont.) Gom. and *Anabaena* sp. Abundant.

In the material at hand the frond is not penicillated as shown in the description of *Phormidium penicillatum* Gom., but is found associating with other Cyanophycean algae. The sheath is very thin and mostly diffluent. The trichome is constantly 6μ in diameter and a variation concerning the dimension is not found. In length the cell shows a range between 6μ and 12μ , from nearly quadrate to 2 times longer than their diameter. This species is new to Japan.

Genus *Lyngbya* Ag. ex Gomont, 1892

- | | |
|--|---|
| 1. Trichomes 1.2~3 μ in diameter | 2 |
| 1. Trichomes 7.8~15 μ in diameter..... | 4 |
| 2. Filaments fixed at the base | <i>L. infixa</i> |
| 2. Filaments fixed for the entire length | 3 |
| 3. Cells 1.2~1.8 μ in length; dissepiments thin, obscure..... | <i>L. epiphytica</i> |
| 3. Cells 1.3~4 μ in length; dissepiments thick, conspicuously pellucid | <i>L. pellucida</i> |
| 4. Sheaths coloured..... | <i>L. aestuarii</i> f. <i>spectabilis</i> |
| 4. Sheaths colourless | 5 |
| 5. Trichomes 7.8~9 μ in diameter | <i>L. semiplena</i> |
| 5. Trichomes 9.3~15 μ in diameter | <i>L. confervoides</i> |

Lyngbya infixa Frémy, Cyan. Côtes d'Europe, Mém. Soc. Sc. nat. et math. de Cherbourg, 41: 110, pl. 30, fig. 1(1934).

Filaments 2.5~3.3 μ in diameter; trichomes 2.3~3 μ in diameter; cells 1~3 μ in length; cell contents finely granular.

Hab. Nakano-shima (Jun. 5, 1953). On *Heterosiphonia pulchra* (Okam.) Fkbg. Abundant. No. 1140 f(in Umez. herb.).

Lyngbya infixa was established basing upon the specimens epiphytic on *Udotea petiolata* (Turra) Boerg. and *Codium tomentosum* (Huds.) Stackh. by P. Frémy in 1932. The trichomes are 1.8~2 (rarely 2.8) μ in diameter, and the cells are 1~2 μ in length, according to Frémy's description. As stated in the above description the trichome is 2.3~3 μ in diameter. Judging from the description and figure of *L. infixa* Frémy the present alga is nearly identified with it, except that the dimension of the trichome is somewhat thicker.

Lyngbya epiphytica Hieron. ex Geitl., Cyan. in Rabenh., Kryptogamen-fl. 14: 1038, fig. 656 d(1932).

Japanese name. Itomakimo (I. Umezaki, 1950).

Trichomes 1~1.5 μ in diameter; cells 1.2~1.8 μ in length.

Hab. Takara-zima (May 27, 1953). On *Lyngbya aestuarii* (Mert.) Liebm. f. *spectabilis* (Thur.) Gom. Abundant. No. 1139 c(in Umez. herb.).

Lyngbya pellucida sp. nov. (Fig. 1)

Fila epiphytica, tote affixa, solitaria; vaginae hyalinae, tenues; trichomata pallide aeruginea, apice non attenuata, ad genicula non constricta; cellula 1.5~2.4 μ crassa, 1.3~4 μ longa; dissepimenta non granulata, conspicue pellucida; contentu homogenero; cellula terminali rotundata, neque capitata neque calyptata.

Filaments epiphytic, attached for the entire length, solitary; sheaths hyaline, thin; trichomes pale aeruginous, not tapering at the ends, not constricted at the cross walls; cells 1.5~2.4 μ in diameter, 1.3~4 μ in length; dissepiments not granulated, conspicuously pellucid; cell contents homogeneous; terminal cells rounded, neither capitate nor calyptate.

Hab. Takara-zima (May 27, 1953). (On *Lyngbya aestuarii* (Mert.) Liebm. f. *spectabilis* (Thur.) Gom. Scarce. No. 1139 e (Type, in Umez. herb.).

The filaments are sparsely found attaching for their entire length on other *Lyngbya*, like a habit of *Phormidium epiphyticum* Gardn. In this material the filament is not spirally curved and not encircling a host alga, but in general short and slightly curved. The dissepiments of the cells are so thickly pellucid that each cell is distinctly recognized.

This species of *Lyngbya* seems closely related to *Lyngbya epiphytica* Hieron. and *Phormidium epiphyticum* Gardn., but differs from these two algae in the facts that the dissepiments of the cells are more apparently pellucid, the trichomes are more thicker, and the filaments are generally more shorter.

Lyngbya aestuarii (Mert.) Liebm. f. *spectabilis* (Thur.)

Gom., Monogr. des Oscill., Ann. Sc. nat. Bot. VII, **16**: 130 (1892).

Filaments densely interwoven, rarely branched; sheaths hyaline on the exterior, but on the interior lamellated and yellowish golden colour, up to 12μ in thickness; trichomes $11.5\sim 15\mu$ in diameter; cells $2.5\sim 4.5\mu$ in length.

Hab. Takara-zima (May 27, 1953). On rocks. Scarce. No. 1139 1 (in Umez. herb.).

Lyngbya semiplena J. Ag. ex Gomont, Monogr. des Oscill.,

Ann. Sc. nat. Bot. VII, **16**: 138, pl. 3, figs. 7~11 (1892).

Filaments up to 1 mm in length, $9.3\sim 12\mu$ in diameter; sheaths up to 3μ in thickness; trichomes $7.8\sim 9\mu$ in diameter; cells $1.5\sim 3\mu$ in length.

Hab. Takara-zima (May 27, 1953). On *Galaxaura* sp. Abundant. No. 1139 g (in Umez. herb.).

The writer has referred this specimen to *L. semiplena* with some hesitation since the filament is much short in length. But other characteristics are typical.

Lyngbya confervoides C. Ag. ex Gomont, Monogr. des Oscill., Ann. Sc. nat. Bot.

VII, **16**: 136, pl. 3, figs. 5~6 (1892).

Filaments 5 cm in length, $12\sim 23\mu$ in diameter; sheaths up to 3μ in thickness; trichomes $9.3\sim 15\mu$ in diameter; cells $2\sim 4.5\mu$ in length.

Hab. Nakano-shima (Jun. 5, 1953). Growing on rocks. Abundant. No. 1140 c (in Umez. herb.).

Genus *Microcoleus* Desm. ex Gomont, 1892

Microcoleus tenerrimus Gomont, Monogr. des Oscill., Ann. Sc. nat. Bot. VII, **15**:

355, pl. 14, figs. 9~11 (1892).

Filaments up to 18μ in diameter; trichomes $1.8\sim 2\mu$ in diameter; cells $3\sim 5.6\mu$ in length.



Fig. 1. *Lyngbya pelucida* Umezaki sp. nov. Three filaments ($\times 750$).

Hab. Nakano-shima (Jun. 5, 1953). Growing among the masses of *Phormidium Corium* (Ag.) Gom. and other Cyanophyceae. Abundant. No. 1140 b(in Umez. herb.).

Genus *Hydrocoleum* Kuetz. ex Gomont, 1892

1. Trichomes 7.5~14 μ in diameter *H. lyngbyaceum*

1. Trichomes 21.5~24.5 μ in diameter *H. cantharidosmum*

Hydrocoleum lyngbyaceum Kuetz. ex Gomont, Monogr. des Oscill., Ann. Sc. nat. Bot. VII, 15: 337, pl. 12, figs. 8~10 (1892).

Trichomes 7.5~14 μ in diameter.

Hab. Takara-zima (May 27, 1953). On *Galaxaura* sp. in company with other Cyanophyceae and on rock. Abundant. No. 1139 f(in Umez. herb.).

Hydrocoleum cantharidosmum (Mont.) Gomont, Monogr. des Oscill., Ann. Sc. nat. Bot. VII, 15: 336, pl. 12, figs. 6, 7(1892).

Trichomes 21.5~24.5 μ in diameter or sometimes up to 27.5 μ ; cells 2.5~4.5 μ in length, 5~11 times shorter than the diameter.

Hab. Takara-zima (May 27, 1953). On rocks. Abundant. No. 1139 b(in Umez. herb.).

In the specimen from Takara-zima there are some trichomes having large dimension attaining 27.5 μ and their cells are very short, being a length of 5~11 times shorter than the diameter. Such characteristics accord very well with those of *Hydrocoleum Holdenii* Tilden. It appears that *Hyd. Holdenii* is to be combined into *Hyd. cantharidosmum*. In 1932 L. Geitler hinted such a validity for combination.

抄 録

トマトの表皮のクチクラ層にある色素の形成と光の影響

[Pringer, A. A. and Heinze, P. H.: Effect of light on the formation of a pigment in the Tomato fruit cuticle. Plant physiol. 29: 467 (1954)]

トマトの表皮のクチクラ層にある黄色色素の出現は光週性をもつていて、外界の光の影響を多分にうけることが判つた。実験材料には Rutgers というトマトの変種の果実をまだ青いうちにとつて、4箇あて選んで箱に入れ、横面を光にさらせるようにして、色々な光の実験に用いた。コントロールは同数を同様に処理し、暗黒で 21° 或は 26.7° で完熟させる。光の効果の度合は、表皮を 2.5 cm 直径にはいでアセトンで抽出し、液の色を、無色 0—橙色 9 の各段階に分けて比較している。

結果として得られたことは

1. クチクラ層の黄色色素は光のもとで完熟したものには出現するが、暗黒下では出現しない。

2. 最も効果のある波長は赤 (5800 Å~6700 Å) である。
3. 1日数分赤色光をあてると黄色色素が出来る。
4. 赤色光の効果はウルトラレッドの光を直後に照射することによつて消失する。
5. 黄色色素はフラボノイドらしい, Rf=0.92 (ethyl ether) 0.68~0.71 (60% isopropyl alcohol)。

1~4 までの結果は *Xanthium saccharatum* の花芽形成, *Lactuca sativa* の種子発芽の折に見られたこととよく符合しており、光週性 (photo-periodic response) は植物体の発生上色々の時期、場所に見られるようになった。(石川茂雄)

Studies on Anthocyanins XXV¹⁾

Paper Chromatographic Investigation on Anthocyanins occurring in the Leaves of *Perilla** varieties

Kôzô HAYASHI and Yukihide ABE**

林 孝三・阿部幸頼： アントシアンの研究, 第 25 報. ペーパークロマトグラフ
によるシソの葉のアントシアンの調査

Received December 24, 1954

Perilla frutescens Britton var. *crispa* Decaisne (Japanese name: Shiso), which belongs to Labiatae, is commonly cultivated in Japan, owing to the excellent quality of the red color applicable for dyeing some foodstuffs. The plant includes several garden varieties, of which the following two are commonly used for the above mentioned purpose: 'aka-shiso' bearing red smooth leaves, and 'chirimen-aka-shiso' having red wrinkled leaves. So far as we know, the coloring matter of the latter variety has been submitted to chemical investigations by two Japanese authors. K. Kondo²⁾ had succeeded in isolating a crystalline red coloring principle, which he called 'perillanin' and to which he gave a structure of delphinidin monoglucoside combined with one molecule of protocatechuic acid. Later C. Kuroda and M. Wada³⁾ arrived, however, at a different conclusion; according to them, the coloring matter consists of *p*-hydroxycinnamoylcyanin chloride, for which they have tentatively proposed the name 'shisonin'. If these two findings were both correct, and there was no error in identification of plants, this discrepancy should be understood only in the sense that this difference may be due to the difference in locality, and hence in the difference in biochemical activity; that is, Kondo's material was collected at Toyama, about 400 km (by railway) northwest of Tokyo, and Kuroda and Wada's, at Tokyo. As was already pointed out by S. Hattori⁴⁾, this is highly doubtful from the physiological and genetical point of view, and it would be of great importance to carry out a careful re-examination in this matter. The present study deals with the characterisation of the red coloring principle contained in 'shiso'-leaves chiefly by means of paper chromatography. For the present, two kinds of plant material were used, *i.e.* 'akashiso' and 'chirimen-aka-shiso', which have been growing in a corner of the experimental field of our institute. The crude leaf extracts from both plants gave the same chromatographic features, when run with various solvent mixtures, as reproduced in Fig. 1. As a rule, six anthocyanin spots could be detected on the two-dimensional chromatogram, run with two kinds of solvent mixture.

* Contribution from the National Institute of Genetics, No. 99

** National Institute of Genetics, Mishima

Among them, three spots (Nos. 4, 5 and 6) seemed to be almost insignificant on behalf of the extreme weakness in tint. The chief anthocyanin of *Perilla*-leaves is, in practice, represented by the largest distinct spot (No. 2); the adjacent smaller two (Nos. 1 and 3) are regarded as derivatives thereof, probably associated with unknown substances.

Next, the chromatograms were run with the crude anthocyanin extracts of both materials after saponification with alkali. Two chromatograms thus obtained proved to be quite identical, and only one single spot, the R_f -value of which agreed well with that of cyanin itself, was found. Accordingly, it is obvious that all six anthocyanin spots observed in crude leaf extracts of both varieties belong to cyanin derivatives.

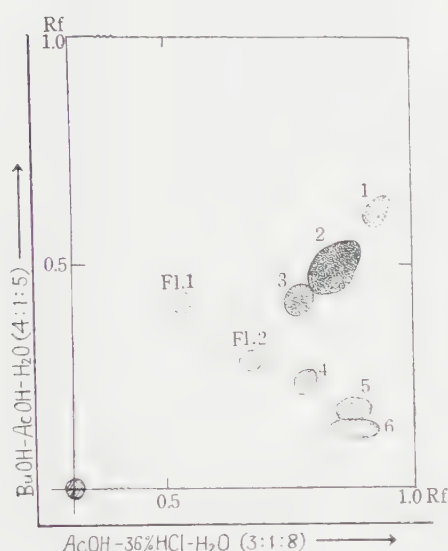
In order to get further conclusive evidence, attempts were made so as to bring the pigment into crystalline form, and the anthocyanins were however isolated, in amorphous state, and seemed to be identical with each other by their appearance. They were then treated with 10 per cent sodium hydroxide solution, and converted into characteristic rhombic leaflets. Identity of these crystals with cyanin chloride was established by paper chromatography and, also, by careful comparison of the melting point as well as by color reactions. The organic acid moiety liberated after this treatment with alkali was found by paper chromatographic method to be *p*-coumaric acid (*p*-hydroxycinnamic acid).

A series of experiments described above convinces us that the red *Perilla*-leaves owe their color not to 'perillanin' but exclusively to 'shisonin', just as pointed out by C. Kuroda and M. Wada. Kondo's 'perillanin' is seemingly due to a wrong interpretation of his own experimental data; his description is wanting of clearness especially regarding an acidic component and the acid-free glycoside.

Experiments

1. **Preliminary test on crude leaf-extracts by paper chromatography.** A handful of fresh mature leaves was collected from each of the two garden varieties, 'chirimen-aka-shiso' and 'aka-shiso' in September, 1954. Dark red leaves were immediately immersed in 400 ml. of cold 1 % methanolic hydrochloric acid. A small amount of each extract was spotted directly on the filter paper (Tôyô, No. 5; 40 × 40 cm), and the chromatographic separation was achieved at room temperature by ascending, two-dimensional procedure, using butanol-acetic acid-water (4:1:5, v/v) in one direction, and acetic acid-36% hydrochloric acid-water (5:1:5, v/v) in the other. Both extracts gave an identical chromatogram, as shown in Fig. 1. The main coloring component is represented undoubtedly by spot No. 2, and the closely related concomitants by two smaller subsidiary spots, No. 1 and 3. The remaining three, Nos. 4, 5 and 6, appear to be negligible on account of their very faint tint. Besides, two small yellowish spots (Fl. 1 and Fl. 2) could be detected, which are probably

Fig. 1. Two-dimensional chromatogram of anthocyanins in *Perilla*-leaves.



of flavone nature.

2. Paper chromatographic test for aglycone. Crude leaf-extracts obtained above were mixed with an equal volume of 20% hydrochloric acid, and heated on a water bath at 95°C for half an hours. After cooling, the hydrolysates were diluted with about 2 volumes of water and extracted with one tenth volume of isoamyl alcohol. The amyl alcoholic layer was separated and spotted on the filter paper, then chromatographed with solvent mixtures such as shown in Table I. The chromatograms obtained from both materials were found to be identical, and gave only one anthocyanin spot, which could be identified with cyanidin chloride.

Table I. R_f -value of the aglycone (cyanidin) from *Perilla*-leaves (at room temp., $28 \pm 2^\circ\text{C}$)

	AcOH-36%HCl-H ₂ O (5:1:5, v/v)	Acetone-10%HCl (1:1, v/v)
'Aka-shiso' (A)	0.36	0.34
'Chirimen-aka-shiso' (B)	0.36	0.34
(A) + (B) + Cyanidin	0.36	—
Cyanidin	0.36	0.34

3. Isolation and detection of cyanin chloride from the two garden varieties of *Perilla*. About 130 g of fresh leaves were necessary for obtaining the smallest amount of crystalline substance. This amount of raw material was thoroughly extracted with 400 ml. of 1% methanolic hydrochloric acid. The filtered red solution was mixed, under continuous agitation, with 150 ml. of 10% methanolic solution of lead acetate. The bluish green precipitate formed was filtered by suction, and immediately converted into chloride by means of 50 ml. of 5% methanolic hydrochloric acid. After filtration, crude anthocyanin was precipitated by the addition of 5 volumes of ether. The amorphous precipitate was dissolved in 20 ml. methanolic hydrochloric acid and again precipitated with ether. Since this product was found to be hardly convertible into a crystalline state, saponification of the pigment was carried out by dissolving it in cold 10% sodium hydroxide solution in an atmosphere of hydrogen. After standing for an hour, the reaction mixture was acidified with hydrochloric acid, and thoroughly extracted with ether. The ethereal solution was evaporated to dryness, and the resultant white crystalline substance was chromatographically

studied. The detection of the acid spot was effected by spraying the diazotized *p*-nitraniline reagent⁶⁾, whereby the grayish blue color characteristic of *p*-coumaric acid was produced. The results are summarized in Table II.

The acidic aqueous layer was concentrated *in vacuo* up to sirup, from which the free anthocyanin was extracted with a minimum quantity of ethanol. After addition of several drops of conc. hydrochloric acid and standing for several days in an ice-box, the dark chocolate-brown crystalline substance separated in a small amount. This was collected and recrystallized once from aqueous alcoholic hydrochloric acid, and obtained in characteristic, brown colored, rhombic leaflets. In this state, the anthocyanin specimens from both plants showed quite identical characteristics in every respect. They melted at 190~191° under decomposition. Also the paper chromatographic analyses proved their identity with cyanin chloride, as is shown in Table III.

Table II. R_f-values of acidic components of *Perilla*-anthocyanins
(Tôyô filter paper, No. 50; 25°C).

	Benzene-AcOH-H ₂ O (2:2;1) ⁶⁾	<i>o</i> -Cresol-AcOH-H ₂ O (50:2:48) ⁷⁾	50 % EtOH
<i>p</i> -Coumaric acid	0.45	0.79	0.91
'Aka-shiso' (A)	0.44	0.79	0.92
'Chirimen-aka-shiso' (B)	0.45	0.78	0.92
(A) + (B)	0.44	0.79	0.92
(A) + <i>p</i> -Coumaric acid	0.45	0.79	0.92
(B) +	0.45	—	—
(A) + Protocatechuic acid	—	0.79: 0.31	0.92; 0.81
Protocatechuic acid	—	0.32	0.81

m-Cresol was used instead of *o*-cresol.

Table III. R_f-values of acid-free *Perilla*-anthocyanins, showing their identity with an authentic specimen of cyanin chloride (Tôyô filter paper, No. 50; 25°C).

	BuOH-AcOH-H ₂ O (4:1:5, v/v)	BuOH-36% HCl-H ₂ O (50:30:55, v/v)	AcOH-36% HCl-H ₂ O (3:1:8, v/v)
Cyanin chloride	0.13~0.14	0.35~0.38	0.54
'Aka-shiso' (A)	0.14	0.38	0.54
'Chirimen-ake-shiso' (B)	0.14	0.38	0.54
(A) + Cyanin	0.14	0.38	0.54
(B) + Cyanin	0.14	0.38	0.54
(A) + (B)	0.14	0.38	0.54
Chrysanthemin	0.25~0.26	0.46~0.48	0.41

Summary

In order to answer the question, which is the essential component of *Perilla*-anthocyanin, 'perillanin' or 'shisonin', leaf extracts from two common varieties of the plant were subjected to paper chromatographic study. The results have shown that the anthocyanin is nothing other than 'shisonin', *i.e.* *p*-hydroxycinnamic acid derivative of 3:5-*o*-diglucosidylecyanidin, as described by Kuroda and Wada. There was found no evidence in favor of the presence of Kondo's 'perillanin'.

We are indebted to Dr. Chika Kuroda for the kind supply of the authentic specimen of *p*-coumaric acid, and to Prof. Shoji Shibata, of the University of Tokyo, for furnishing us with protocatechuic acid.

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抄 録

チサの種子の発芽に及ぼす光の作用

[Borthwick, H. A., Hendricks, S. B., Toole, E. H., and Toole, V. K.: Action of Light on Lettuce-seed Germination. Bot. Gaz. 115: 205 (1954)]

種子の発芽に光の波長が関係する事は Flint, McAlister (1935), Resihr (1939) 等でみられている。Flint, McAlister はすでにチサの発芽に赤色光が有効で、青色光及び赤外線は抑制的に作用することをみた。著者は更に詳細な action spectra を測定して、6400~6700 Å (赤色光) の波長部分が最も鋭敏に発芽を促進させ、7200~7500 Å (赤外線) の波長部分は発芽を顕著に抑制し、又 4000~5000 Å (青色部) の間には非常に弱い促進と抑制部分があるらしい。この種子の action spectrum は花芽形成の際の光週期の action spectrum と非常に類似している事は注目に値する。

この種子中に存在する2つの色素物質即ち、赤色光を吸収する色素物質と、赤外線を吸収する色素物質は照射温度に関係なく相互に赤色光及び赤外線を吸収して可逆的に変換し、それぞれ前者は赤外線を吸収する色素物質に、後者は赤色光を吸

収する色素物質になる。この変換は何回でも繰り返して行うことが出来、この光化学反応はチオイソデゴールの光化学シス、トランス異性化反応の様なものかも知れない。

又種子を一定時間間で浸漬した後赤色光を与えこれを闇中で温度を 30°C 以上にすると発芽が抑制され、これに再び赤色光を与えて 20°C の闇中に置くとほとんど完全に発芽する故闇の中で赤外線を吸収する色素物質を再び赤色光を吸収する色素に変える光化学反応とは異つた反応系がこの種子の中にある。これと類似の反応には花芽形成の際の光週期間の闇期が或程度以上長くなると花芽形成が抑制される場合の反応がある。

又種子を浸漬後に赤色光を与え室温 (20°C) に保つと、それに続く発芽の方向への反応が進行し次第に可逆性を減じ或一定時間 (10 時間) 後には赤外線を与えても早や発芽は抑制されない。

(下河原五郎)

Abnormal Growth in *Allium monanthum*

by Yudzuru OGURA

小倉 謙: ヒメニラの異常成長

Received January 14, 1955

Some years ago, Mr. K. Hisauchi proposed to the writer some specimens of *Allium monanthum* Maxim. provided with long stolons. Based on these specimens and other materials obtained hereafter near Tokyo, as well as on materials under culture, the writer could trace the mode of growth and formation of the stolons. As the formation of such a long stolon in allied species has never been described, the writer expects to describe the results obtained here.

External Form

Near Tokyo, this plant begins to germinate in the middle of March, blossoms at the beginning of April, and leaves and flowers wither away at the beginning of May,



Fig. 1. *Allium monanthum*, showing the sterile and fertile individuals. $\times 3/5$



Fig. 2. *Allium monanthum*, showing individuals with stolons. Central two, individuals with short stolons, collected in the field; below, individual with long stolon, cultivated, short secondary stolon is shown. $\times 3/5$

* Contributions from the Division of Plant Morphology, Botanical Institute, Faculty of Science, University of Tokyo, N. S. No. 65.

Read at Section 8 in the 8th International Botanical Congress at Paris, July 8, 1954.

leaving only small bulbs under the ground, which germinate next spring.

This plant is much smaller and more simplex compared with other species of *Allium* (Fig. 1). The bulb is small spherical or ovoid in shape, 3~5 mm in diameter. The surface is covered with a soft dark-brown scale, and at a point of the lower surface numerous thin, but long roots are attached. The upper part of the bulb elongates gradually into a leaf stalk, which extends as one or two green foliage leaves. Each foliage leaf is 5~10 cm in length and 3~5 mm in breadth. In the fertile individual, a thin floral stalk springs out of the leaf stalk, and usually only one small flower is attached on its tip, though there are some cases provided with two or more flowers.

Among these individuals showing normal forms, we find abnormal ones provided with stolons gone out of the old bulb (Fig. 2). The stolon is thin, and its length is very variable, 1~30 cm. It creeps usually horizontally under the ground and white and soft, but in the field, if it is exposed more or less on the ground, it becomes greenish and hard (Fig. 2, two central). The tip of the stolon swells out in an ovoid form and on this swollen part a green foliage leaf of a normal form goes out, but no roots are formed. Rarely the second stolon is found grown out of this swollen part (Fig. 2, below).

Structure

In the normal sterile individual (Fig. 3, A), the bulb consists of an outer dark-brown scale, a thin white scale and inner two or three thick white scales, the outer one enclosing the inner one in turn. One or two of the thick scales elongate into long stalks, whose tips expand as one or two foliage leaves. The bulb is, therefore, a simplified bulb consisting of a few enclosed scales, as seen in other species of *Allium* and

many other Monocotyledonous species, the inner thick scales representing the bulblet, which germinates next spring. Moreover, we see usually a small bulblet formed within the scale, which represents an adventitious bulblet, which germinates also next spring.

In the normal fertile individual (Fig. 3, B), the bulb consists, just as in the sterile, of an outer dark-brown scale, a thin white scale and two or three thick white scales, but a small branch is found just at the outside of the innermost scale, and

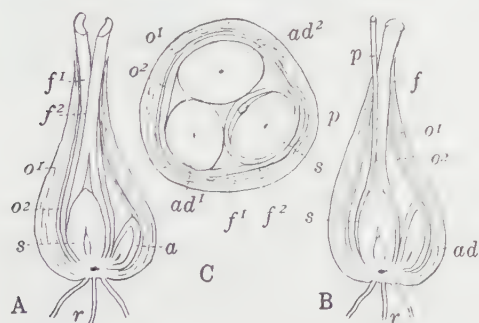


Fig. 3. Longitudinal and transverse sections of the bulb, showing its construction. A, sterile; B, fertile; C, fertile with two adventitious bulblets; o₁, o₂, outer scale; f₁, f₂, scale whose upper part transforms into foliage leaf; s, inner scale; p, peduncle; ad, adventitious bulblet; r, root. $\times 5/2$

passing through the leaf stalk it becomes a peduncle, on which one, sometimes more

than two flowers are produced. Within the bulb are found also one, rarely two or more adventitious bulblets (Fig. 3, C).

In either sterile or fertile individual, the normal and adventitious bulblets within the bulb grow out during the spring, and after a long sleeping, they germinate in the next spring as new individuals.

In the individual with the stolon, the structure is different from the normal. The mother bulb, from which the stolon is produced, consists of a dark-brown scale only, and it is shelling and is empty including no fresh scales, and the stolon, sprung out of the base, where the roots are attached, passes through the scale and runs out horizontally.

The stolon is thin, 1 mm in diameter and is traversed longitudinally by five vascular bundles. It is covered by a very thin, transparent scale throughout. The swollen part of the stolon (Fig. 4) consists of the scale with an empty cavity, in which always two small bodies, similar in form and size, are enclosed. The upper part of the cover elongates into a leaf stalk, which expands as a foliage leaf. Two

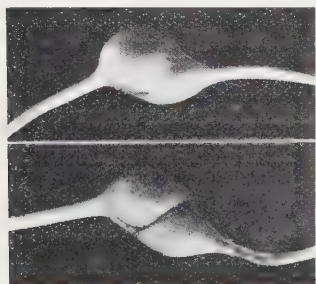


Fig. 4. Swollen part of the stolon, showing surface and internal structure. Below, removed the scale to show two bulblets. $\times 2$

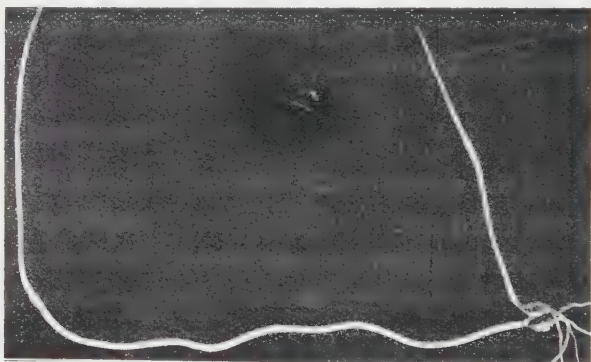


Fig. 5. Germination of the swollen part of the stolon, the one germinating *in situ* and the other germinating into a stolon, in one part of which a small swollen part is seen. $\times 3/5$

bodies are white or greenish and consist of outer thin scale and thick inner ones, and represent the structure of the bulblets. One of the two is always sessile, while the other is provided with a short stalk, and the former corresponds to the main bulblet, while the latter the adventitious. The construction of the swollen part shows, therefore, that of a bulb including an adventitious bulblet.

Growth of the Bulb and Formation of the Stolon

In order to know the growth of the bulbs and the development of the stolons, it is necessary to cultivate them, as the stolons creep to an unexpected direction under the ground. If we cultivate the individual with a stolon, the older dark

brown scale, the stolon and the foliage leaves wither out in the beginning of May, leaving only the swollen part of the stolon, which during this period grows up, but no roots are produced. In the next spring two bulblets within it germinate (Fig. 5), the one as a normal sterile individual with one or two foliage leaves upwards and numerous roots underneath, while the other elongates into a new stolon, and one foliage leaf is produced on its tip, and the base of the foliage swells out gradually to become a small bulb. The stolon creeps to any direction straightly or curving to and fro. As described before, the adventitious bulblet included within the swollen part has a short stalk, and it is this stalk which elongates into the stolon partaking the bulblet on its tip. This mode of growth is invariably repeated (Fig. 6). It is

rarely found that the adventitious bulblet within the swollen part of the stolon has a long stalk, so that the small stolon breaks out of the cover of the swollen part and carries a foliage leaf on its tip (Fig. 2, below).

If we cultivate the normal bulb without stolon, two or three individuals germinate from it in the next spring (Fig. 6, A-B). In the case of two individuals, the one germinates in the original place, while the other is apart from there, but is connected by a stolon. This is case, when there is only one adventitious bulblet, and this mode of growth is coincided with that of the swollen part of the stolon. In the case when three individuals germinate, the one is normal, while the other two are situated on the tips of the stolons. In the field, as the mother bulb withers out, it is difficult to trace the natural situation of new individuals.

The mode of formation of this long stolon in this species is very peculiar, as the same phenomenon has never been known in allied species, but if we consider

the allied species, we can understand this phenomenon. Apparently the stolon formation is alike to the dropper formation of *Tulipa* species, but really both of them are quite different in the mode of formation, as has been described by the writer before*.

* Ogura, Y. Morphology of the subterranean organs of *Erythronium japonicum* and its allies. Phytomorphology. 2: (1952).

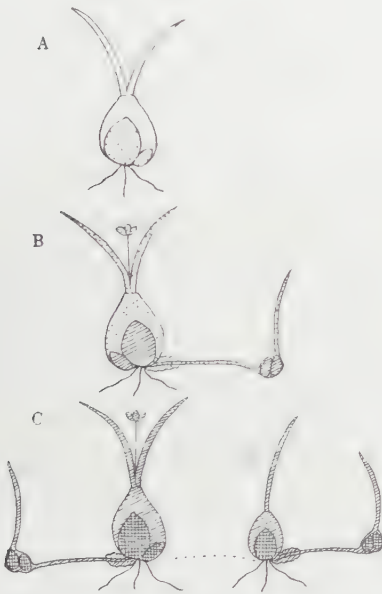


Fig. 6. Diagrams showing the mode of growth of the bulb and formation of the stolon. A, first year (white), including bulblets for next year (dotted); B, second year (dotted), including bulblets for next year (lined); C, third year (lined), including bulblets for next year (crossed).

It is not curious in other species of *Allium* and other species of Liliaceae that, within the bulb one or more bulblets are formed, which germinate as new individuals crowded with each other, and it is also found in some cases, such as *Allium nipponicum* or *Ornithogalum umbellatum*, that each bulblet is provided with a short stalk at its base, which remains shortly even after germination. The writer considers that, if the stalks of these bulblets in these species elongate themselves, the formation of the long stolons will be resulted as in the present species. Moreover, in other species as the bulb is large the bulblets within it are numerous, while in the present species, as the bulb is small consisting of a few scales, the bulblets within it are only one or two. The swollen part of the stolon corresponds therefore, to one of adventitious bulblets within the mother bulb in other species.

The curious mode of formation of the stolon in *Allium monanthum* is convenient for the vegetative propagation, and it is very interesting that most of the flowers of this species are sterile. Really, in the plants growing near Tokyo, most of the flowers are female only. The sterility of the flower and the formation of stolon may have a close correlation.

Summary

1. In *Allium monanthum*, the bulb consists of a few enclosed scales, and includes usually one adventitious bulblet.

2. The latter germinates in the next spring into a long stolon, the tip of which swells out and produces a foliage leaf. In this swollen part usually two bulblets are produced, one of which germinates in the next spring into a stolon.

3. The formation of the stolon is repeated year by year, and it may be adapted for the vegetative propagation.

摘 要

ヒメニラは小さい宿根草で、地下に鱗茎があり、これから小さい1~2葉と1本の花茎を出し、その上に通例1花をつける (Fig. 1)。しかるにある個体には鱗茎から細長い匍枝が出て水平に走り、その先が膨らみその上に1葉をつけるものがある (Fig. 2)。普通型のものと匍枝を有するものとの発達過程を知ることは現地では困難なので、栽培実験によつて確めた。

その結果を要約すれば (Fig. 6)、普通個体 (A, 白で示す) には翌年用の小鱗茎の外に1個の不定小鱗茎 (点で示す) を含み、翌年この2鱗茎は

夫々成長するが1はその位置で普通の個体となるが、1は細長く伸びてその先が膨らみその上に1葉をつけるが (点で示す) 普通鱗茎及びこの匍枝上の膨らみの中にも2小鱗茎 (線で示す) が生じ、その翌年に至り夫々普通型及び匍枝を有する個体 (C, 線で示す) に発達し、各々に夫々2小鱗茎が生ずる (クロスで示す)。

以下これを繰返すので、この匍枝により、原体から遠いいろいろの方向に新しい個体を分布することができ、これはこの種の營養繁殖に適應した形質といえよう。

A Brief Note on the Sugars of *Lycopus lucidus*

by Kiyoshi NAKAHARA*

中原清士： シロネの糖について

Received January 13, 1955

In the stolon of *Lycopus lucidus* Turczaninoff a crystalline hexasaccharide, lycopose, was reported to be present by Murakami¹⁾, who showed that it is composed of 1 *M* of fructose and 5 *M* of galactose and assigned to it the structure pentagalactosido-fructoside. Murakami further found a hexasaccharide, ajugose²⁾ from the roots of *Ajuga nipponensis* Makino, to which a tentative structure galactosido-galactosido-galactosido-glucosido-fructoside was given. This hexasaccharide most probably belongs to the series of raffinose, stachyose, and verbascose³⁾. On the other hand, lycopose differs from ajugose by the absence of glucose molecule. I have been able to confirm the presence in this plant of sucrose, raffinose, and stachyose in addition to glucose, galactose, and fructose. This feature of this plant fairly coincides with that of *Stachys tuberifera*¹⁾ except the presence of lycopose. It is very interesting to note that *Lycopus* plant does contain besides lycopose some oligosaccharides which have no intimate structural relationship. The presence of free galactoside in this plant as in *Stachys* is also noteworthy in that it has hitherto not yet reported in literature, as far as concerned.

Ajugose was considered to be a reserve carbohydrate by the fact that in the roots of *Ajuga* it was present in abundance in winter season. The present study has been undertaken in order to see if lycopose be subjected to any seasonal change and at the same time, whether, sugars other than lycopose be found along with the latter. The method of paper-chromatography was used throughout this study.

Experimental

Material—The plants collected early in August were divided into 4 parts, namely leaf, stem, root, and stolon, but in winter there remain only stolons, so that the investigation was confined solely to this part. Part of the material used in the present study was collected in a suburb of Tokyo and at Karuisawa, Nagano Prefecture, and others were kindly given by Mr. M. Togashi and Prof. K. Hisauchi; some of them have been planted in the garden of this laboratory.

Extraction of the material—10~20 g of the fresh material were crushed with ethanol using Waring Blendor, and extracted with 5 times its weight of 80% ethanol

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on a boiling water-bath. The extraction was repeated with 10 times its weight of 80% ethanol until the extract no more showed the carbohydrate reaction with the trichloroacetic acid-benzidine reagent when tested on a piece of filter paper. The ethanol was then distilled off and to the residual aqueous solution aqueous lead acetate was added. The precipitate was filtered off and the filtrate was treated with hydrogen sulfide. The hydrogen sulfide was driven from the filtrate by the bubble of air and the solution was condensed under reduced pressure after neutralization with dilute ammonia.

A small amount of active charcoal was applied, when necessary, to remove the brownish color of the concentrate. In this case, the amount of charcoal was previously determined in order not to adsorb the sugars besides the colored impurities. This procedure seemed important to get well-formed spots on the paper chromatogram.

Paper partition chromatography—Two sorts of filter paper, No. 2 and No. 50 of the Toyo Filter Paper Co. (Tokyo) were used. Butanol-acetic acid-water (4:1:1)⁵⁾ and phenol-water (4:1) were used as solvents. In most cases the ascending method was adopted. Sometimes especially to achieve good separation and well-formed spots, the chromatographic procedure with butanol-acetic acid-water solvent by ascending method was repeated twice or thrice. As standard sugars, Merck's and Shering-Kahlbaum's preparations of fructose, glucose, galactose, sucrose, lactose, and raffinose were used. Samples of stachyose and lycopose were supplied by Dr. S. Murakami.

For the detection of reducing sugars Horrocks' benzidine reagent⁶⁾ and for the detection of ketose and ketose-containing oligosaccharide Seliwanoff's reagent⁷⁾ was used as usual.

Trichloroacetic acid-benzidine reagent (0.5 g. benzidine, 10 ml. glacial acetic acid, 10 ml. 40% (w/v) trichloroacetic acid, 80 ml. ethanol)¹⁰⁾ was also used for the detection of glucose and glucose-containing reducing and/or non-reducing oligosaccharide.

For the separation of each sugar, the large scale filter paper chromatography⁸⁾ was employed.

Results

Glucose and galactose were detected in all parts of the plant. These two sugars were well separated on the chromatogram run with 80% phenol, as shown in Fig. 2. Fructose was present only in the stolons (Fig. 1). All these three monosaccharides were contained in the stolons even in December, although in a very low concentration.

In addition of these three monosaccharides, there were detected four spots of smaller R_f values (Fig. 1), that is, 0.14, 0.04, 0.015, and 0.00, respectively.

The first spot (R_f 0.14) coincided with sucrose, and the acid hydrolysis products were composed of glucose and fructose almost in an equal quantity (Fig. 3 a, b).

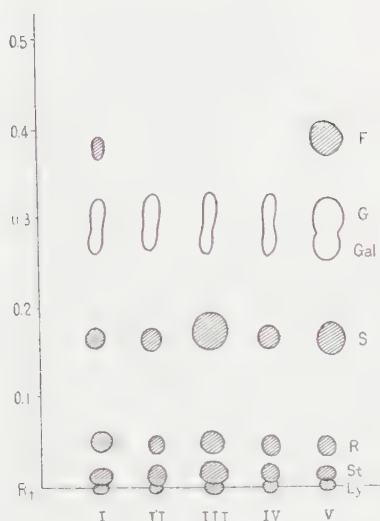


Fig. 1. Chromatogram of the sugars of *Lycopus lucidus* (butanol-acetic acid-water). F, fructose; G, glucose; Gal, galactose; S, sucrose; R, raffinose; St, stachyose; Ly, lycose. The shadowed spots denote positive Seliwanoff's reaction, and the clear circles, positive benzidine reaction. I, stolon; II, root; III, stem; IV, leaf; V, control.

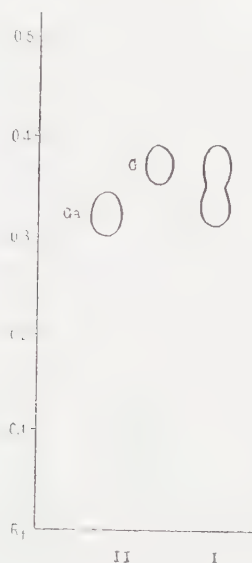


Fig. 2. Chromatographical separation of galactose from glucose with phenol-water (4:1) (I). II, controls.

The R_f (0.04) of the second oligosaccharide was identical with raffinose. When it was subjected to mild hydrolysis with 20 % acetic acid for 4 hours on a water bath, two spots were obtained, one of which corresponding to that of fructose. The R_f value of the other is similar to that of the melibiose, obtained by similar procedure from the authentic raffinose (Fig. 4 a). This latter spot showed positive reaction with benzidine reagent and negative one with Seliwanoff's reagent, an indication that it is an aldehydic sugar. After the separation of this component from the paper chromatogram followed by acid hydrolysis: with NH_2SO_4 , two sugars, identical with glucose and galactose, respectively, were obtained on paper chromatogram in an almost equal quantity. From these results the spot is no doubt identical with raffinose.

The R_f value of the third oligosaccharide was very similar to that of authentic stachyose. This was partially hydrolysed with 20% acetic acid, and the hydrolyzate was chromatographed. As control, authentic stachyose was likewise hydrolysed and chromatographed side by side. There were seen two spots, one of them representing fructose and the other manninotriose. On elution and re-chromatographing, the R_f values agreed completely (Fig. 5 a, b, c). The spot corresponding to manninotriose failed to show any positive reaction with Seliwanoff's reagent, but was positive to benzidine reagent. It was eluted and completely hydrolyzed with NH_2SO_4 for 6

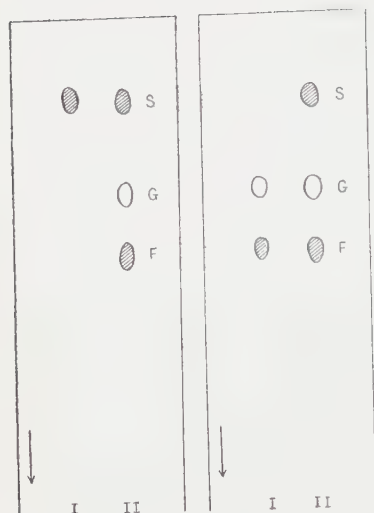


Fig. 3a

Fig. 3b

Fig. 3. a) Separation of the disaccharide, showing the same R_f as that of the authentic sucrose (I). Descending chromatogram.

b) Hydrolysis products of the disaccharide. (I) Chromatographed by the descending method, at 23° for 16 hours.

F, fructose; G, glucose; S, sucrose. II. control.

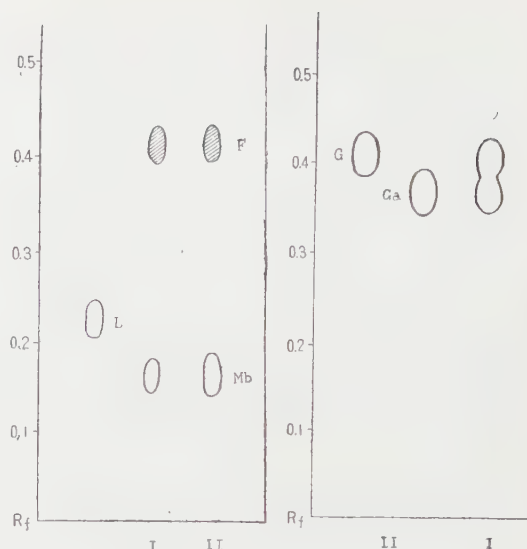


Fig. 4a

Fig. 4b

Fig. 4. a) Chromatogram of the partial hydrolysis products of L_3 (I).

II, control: partial hydrolysis products of the authentic raffinose (run with phenol-water). L, lactose; F, fructose; Mb, melibiose.

b) Chromatogram of the hydrolysis products ($N-H_2SO_4$) of the fraction representing melibiose (run with phenol-water) (I). II, controls.

hours on a water bath. The products proved to consist of glucose and galactose in an approximate ratio of 1:2. In this case, the quantitative comparison between glucose and galactose was made by comparing the diameters or the areas of their spots with those of authentic ones⁹⁾ (Fig. 5d). The hydrolytic procedure with NH_2SO_4 proved to have no effect on the comparative quantities of glucose and galactose, because a mixture of authentic glucose and galactose in a ratio of 1:2 gave consistent results.

The third oligosaccharide did not now show positive benzidine reaction, but was positive against Seliwanoff's reagent. All these facts may be sufficient enough to identify this third spot with stachyose.

The fourth spot, the R_f value of which is practically 0, is to be considered as lycopose. There is at present no procedure available for separating this from stachyose, so that it could not be made clear, what kinds of sugars and how many moles of them are concerned in building it up. The task is reserved for further investigation.

The kind advice and encouragement of Dr. S. Hattori, Prof. of the University of Tokyo, are gratefully acknowledged. The author is also indebted to Prof. S.

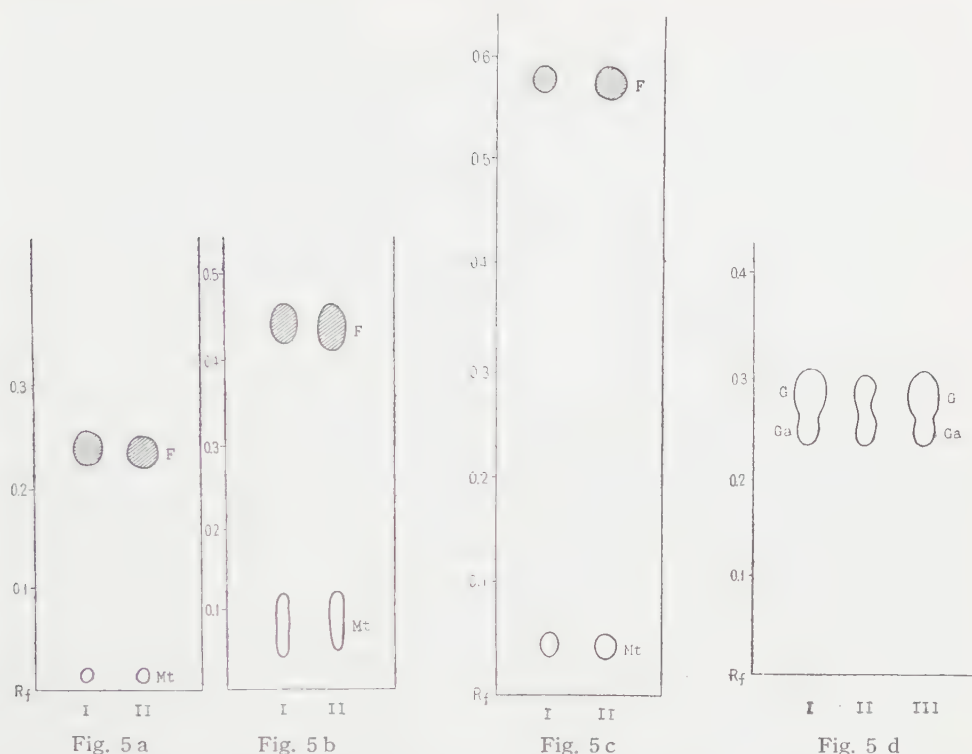


Fig. 5. Chromatograms of the partial hydrolysis products of L_4 (I). Control: partial hydrolysis products of the authentic stachyose (II). a) Chromatographed with butanol-acetic acid-water. b) Chromatographed with phenol-water. c) Chromatographic procedure was repeated three times; the solvent front ascended each time a distance of 29 cm from the starting point (butanol-acetic acid-water). The spot representing fructose moved 16.4 cm, while the fraction representing manninotriose moved only 1.3 cm. d) Chromatogram of the hydrolysis products (I, III) of the manninotriose part of L_4 (run with phenol-water). control (I): the hydrolysis products of the manninotriose, obtained from authentic stachyose. F, fructose; Mt, manninotriose; G, glucose; Ga, galactose.

Murakami, of the Saitama University, to Prof. K. Hisauchi, of the Toho University, and to Mr. M. Togashi of the Takeda Pharmaceutical Company, Osaka, who very kindly furnished me the materials.

Summary

1. The sugar in *Lycopus lucidus* Turczaninoff were investigated by means of paper partition chromatography. 2. Glucose and galactose were present in all parts of the plant. Fructose was confined only in the stolon. 3. In addition to the monosaccharides and a hexasaccharide lycopose, sucrose, raffinose, and stachyose were found.

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On *Cheiropleuria bicuspis* var. *integrifolia*, with Special Reference to the Structure of the Leaf*

by Yoshitomo Nozu**

野津良知: スジヒトツバの形態学的研究, 特に葉について

Received January 16, 1955

The genus *Cheiropleuria* contains a single species, *Cheiropleuria bicuspis* var. *integrifolia*, which was transferred by Nakai (1928, '33) from the Polypodiaceae to his new family Cheiropleuriaceae, basing chiefly upon its particular characteristics of gametophyte and the special type of the development of stomata of the leaf. This species distributes widely in the warmer regions of the Far Eastern territories including the south-west part of the Japan Archipelago. It is a protostelic fern of moderate size, characterized with fairly thick creeping rhizome and long-petioled, dimorphic leaves, i. e., broader sterile ones which are frequently forked at their tip and divoid midrib, and narrower lanceolate fertile ones with distinct midrib. Notwithstanding all of these features are thought to be very interesting, few is known on its morphological and anatomical natures, excepting a certain number of brief notes chiefly on the structure of rhizome (Bower, 1915; Ogura 1938).

In the present study, the writer dealt in detail with anatomical and morphological studies on the adult as well as the juvenile plants, placing a special importance on the venation of leaves.

Material and Methods

The materials used in the present study were collected by the author in Aug. 1950, in Hachijo Island. Paraffin sections were made for the detailed study of the leaves. Materials were fixed in F. A. A., dehydrated in an ethyl-buthyl alcohol series and sections were stained in Heidenhain's haematoxylin. For the study of venation, living leaves without any treatment serve sufficiently for purpose. Sometimes, leaves are boiled in dilute NaOH solution in order to trace vascular skeletons.

Observation

External features

The rhizome is creeping, and surrounded by petiolar base of closely arranged leaves, both being covered densely with brown hairs. Some of the marked charac-

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teristics of the external features of the *Cheiropleuria* seem to be in the rhizome, which is relatively thick and short, 5~8 mm in diameter, branching very rarely, and grows very slowly every year. It shows a distinct dorsiventrality in that the leaves are closely arranged on its dorsal side. Leaves are clustered as in the case of *Osmunda* and the petioles are almost erect, slender and long, and are not articulated at their base. They attain mostly 20~40 cm in length. The lamina of sterile leaves is mostly forked at their top and has no midrib, the main veins branching dichotomously at the lamina base. The fertile leaves are generally smaller and more slender than the former. The lamina has the midrib and the main veins is divided into three at its base. The sori and capitate paraphyses cover densely all over the lower surface.

The roots are relatively thick, long, brownish in colour and branch very rarely. They are arranged not only on the ventral side of the rhizome, but also near each petiolar base on the dorsal side. The arrangement of the roots are relatively regular.

Internal structures

Rhizome. The structure of the rhizome, as has been studied already, is rather simple (Fig. 1, A). Within the epidermis, which is of normal structure, is found the cortical tissue composed mostly of the sclerenchymatous cells, namely, many layers (sometimes more than 20 layers) on the outer side are composed of relatively large cells, while those of the inner layers (4~6 layers) are smaller. This condition resembles to the case of *Gleichenia*.

The protostele is characterized by the narrow phloem and rather massive xylem which is composed of tracheids intermingled with thin-walled parenchyma, just as in the case of *Gleichenia*. At a short distance from the periphery of the xylem, is found variable numbers of small protoxylem masses which are arranged chiefly on the dorsal half of the stele of the creeping rhizome. The single leaf trace issued forth from the rhizome is elliptical and provided with two protoxylem groups.

Leaves. In a cross section, the outline is crescent at the petiolar base and it becomes circular at the upper part of petiole. In the cross section, there 2-3 layers of the thin-walled parenchymatous cells which are followed by the inner layers of the smaller sclerenchymatous cells. There are a pair of elliptical meristemes. The vascular bundle belongs to the *Marattia*-type described by Ogura (1938).

One of the distinct characteristics of the leaf in *Cheiropleuria* is the presence of the stelar system of the *Cheiropleuria*-type as was shown by Ogura (1938). The leaf trace departs dichotomous-sympodially from the protostele of the rhizome and it is soon divided into two at the very base of the petiole (Fig. 1, B, C). This division is true-dichotomy and takes place radially to the axis. At the lower part of the petiole each of the two strands is subdivided into two (Fig. 1, D). At the middle part of the petiole inner two of them are temporarily fused into one which is soon separated again into two, thus there being four strands at the upper part of the petiole (Fig. 1, E).

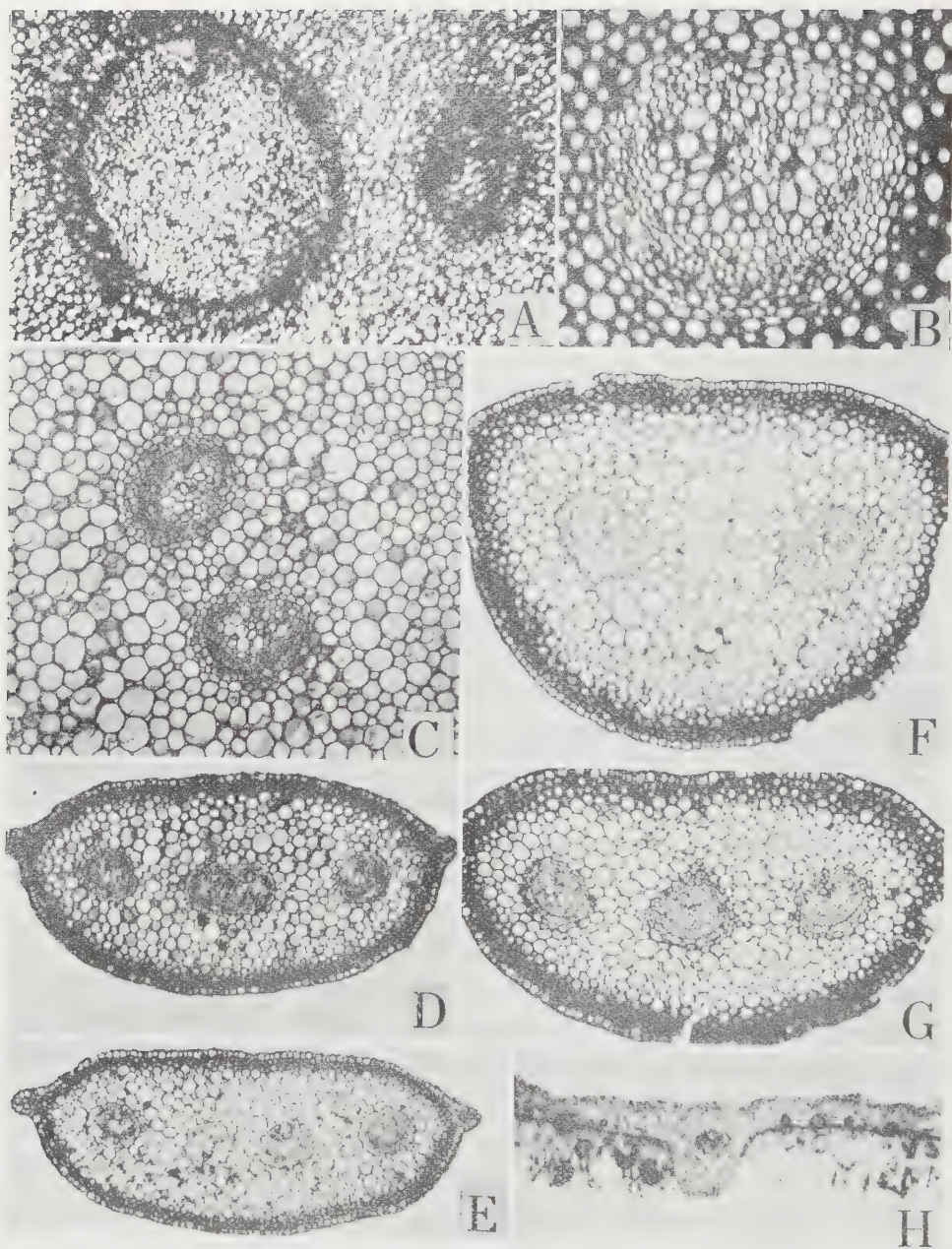


Fig. 1. A, Cross section of the rhizome, showing the stele and the cortex with the leaf trace. $\times 45$; B~E, successive cross sections of the petiole. B; extreme base of the petiole. $\times 100$; C, base of the petiole. $\times 80$; D, middle of the petiole. $\times 50$; E, top of the petiole. $\times 50$; F~H, successive cross sections of the petiolar base (F), middle of the petiole and the lamina (H) of the fertile leaf. $\times 70$

In the case of fertile leaf the vascular system below the middle part of the petiole is similar to that of the sterile (Fig. 1, F, G). Three strands of the middle part of the petiole, however, run invariably into the lamina, the median one of them marking a midrib (Fig. 1, H).

The lamina of the sterile leaf is thick and leathery, about 1.1 mm in thickness. Under the upper epidermis, is found a layer of large cells. The mesophyll consists of undifferentiated roundish cells which are roughly arranged on the lower side. The

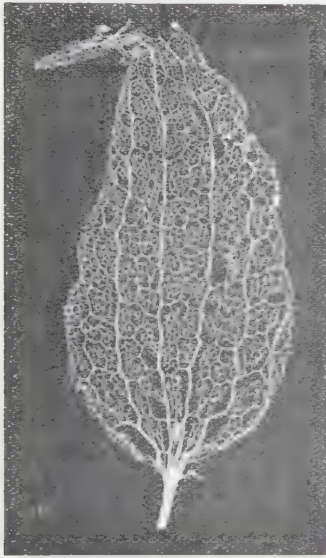


Fig. 2. Vascular skeleton of the lamina of the sterile leaf which shows the main veins and the smaller veins showing the "Venatio Anaxeti". $\times \frac{2}{3}$

prominent veins are four to eight in number and small veins connect each other into a network to make a "Venatio Anaxeti" type. The vascular system at the base of the lamina of the sterile leaf is shown in Fig. 2, which is obtained from a leaf after the treatment of a dilute solution of NaOH. The four main veins at the base of the lamina divergo to extend all over the lamina.

Sorus. The sori cover all over the lower surface of the fertile lamina. Soral areas are made by the patches of receptacles. The sporangium is provided with an oblique annulus consisting of about 18 thick-walled cells and a stalk of four rows of cells. The latter bears capitate paraphyses. The spores are tetrahedral or sometimes bilateral, and their surfaces are smooth.

Roots. Their structure is of typical fern type. Two or three outer layers of the cortical tissue are composed of thick-walled cells, and

5~7 inner layers consist of thin-walled cells. The central cylinder is trirch and surrounded by very distinct endodermis.

The young plant. Successive leaves on several young plants are preferred in order to investigate the origin of the venation.

The first leaf is short petioled and spoon-like in form. The venation is very simple, namely, a single trace which enters into the base of the leaf runs toward the upper part without branching (Fig. 3, A). In the next leaf whose petiole is more or less elongated, the veins shows a dichotomous branching at the upper part of the lamina (Fig. 3, B). In the third leaf the

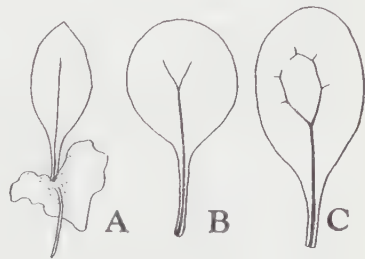


Fig. 3. Successive leaves on several young plants, showing the venation which is peculiar to the species. A, first leaf; B, second leaf; C, third leaf. $\times 2$

lower part elongates into a slender petiole and venation shows the type which is peculiar to the species (Fig. 3, D).

The structure of the first leaf is simple. The petiolar base is cylindrical and its top and the lamina are flat. In the petiole, within the epidermis consisting of thin-walled cells, is seen the cortex of 3-4 layers which consist of thick-walled cells.

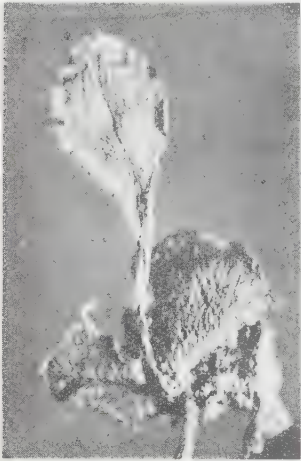


Fig. 4. The gametophyte with the first leaf and the first root. $\times 15$

The bundle surrounded by an obscure endodermis consists of the phloem and the xylem composed of tracheids of from two to three cells. In the second leaf, at a short distance below the level where the bundle of the lamina is dichotomously divided two protoxylem are observable. The structure of the lamina in cross section is composed of unifferentiated, loosely arranged parenchymatous cells which are nearly the same in form and size.

On a young plant which bears the first leaf only, the writer could fortunately find the gametophyte though it was nearly perished, and in the bad state of preservation. The gametophyte is about 5 mm in length and cordate at the base; the midrib is 8-10 cells thick; endophytic fungi are the archegonium is long and large.

Discussion and Conclusion

Cheiroleuria bicuspis var. *integrifolia* was first noted by Blume in 1828. A little later, it was transferred by Presl (1849) to his monotypic genus *Cheiroleuria*. In *Natürlichen Pflanzenfamilien*, Diels (1902) placed this genus in Acrosticheae-Platyserinae, and this classification has been used by Christensen (1906). Bower (1915), who discussed the affinities of ferns from the phyletic point of view, pointed out that this species had mixed characters which were comprehensible as primitive and advanced, and that, in this regard, *Cheiroleuria* should be placed between *Dipteris* and *Platyserium*. But his phylogenetic consideration was apparently established only on the basis of the studies on the sporophyte. On the other hand, Nakai (1928, 33) studied gametophyte and the development of stomata and found that the antheridia were alike to that of the Polypodiaceae, while the archegonia were rather alike to that of the Hymenophyllaceae and Schizaeaceae. He pointed out also that in the presence of mycorrhiza it resembled to the Marattiaceae, Osmundaceae, and Gleicheniaceae, and in the development of stomata, to the Cyatheaceae. In *Genera Filicum*, Copeland (1947) recognized 65 genera of the Polypodiaceae and the genus under investigation was treated as one of the primitive genera on the basis of the characters of sterile and fertile leaves.

One of the characteristic features of the rhizome is the protostelic structure of

the stele, as was already observed by Bower (1915) and the other authors. Bower stated that the stele is of considerable size, and in structure and in form it resembles closely to that of the protostelic *Gleichenia*, such as *G. flabellata*, or *dichotoma*.

This condition is apparently rather uncommon among the living ferns, being restricted, besides *Gleichenia* just mentioned, to *Lygodium*, as well as members of the Hymenophyllaceae (Nozu 1950). Though it will be unnecessary to discuss here in detail, it should be noted that there is an apparent dorsiventrality in the structure of the protostele as shown by the dominant number of the protoxylem groups on its dorsal half. The presence of two groups of protoxylem at the very base of leaf trace is also important.

In this species, the leaf trace originated from the rhizome shows no true dicotomy, but a dichotomous sympodial largely influenced by the monopodial character of the rhizome. The leaves of this species arise more closely than in the case of the protostelic *Gleichenia*. On the other hand, these facts seem to offer some important influence on the construction of the leaf. A similar condition is also found in many Hymenophyllaceae (Nozu 1950), as well as in *Botryopteris cylindrica*.

The size of a leaf trace of *Cheiropleuria* is nearly identical with that of the stele of the rhizome. Thus, it may be held that the ferns which have an actually primitive character become entertained of morphological equivalence of the vascular axes in leaf and rhizome as was already discussed in other ferns by a certain number of authors (Tansley 1908, Campbell 1921, Zimmermann 1930, and Chrysler 1945).

Another characteristic of the species lies in the peculiarity of the stelar system of the leaf, that is, a single leaf trace forks, at the very base of the petiole, into two meristeles which increase their number into four to six by further bifurcations. Each meristele at the petiolar base of this species apparently belongs to the *Marattia*-type described by Ogura (1938). In many species belonging to the *Marattia*-type, however, the meristeles are many in number and the large two on the adaxial side are usually fused sooner later to show the *Onoclea*- or *Asplenium*-type. But in this species, two elliptical meristeles in the petiolar base not change into *Onoclea* and *Asplenium*-type, and the vascular system resembles at a glance to that of *Platycerium* (Bower 1915). Though the latter resembles to the so-called *Polypodium*-type (in the wide sense), they are different in that, in the latter type the abaxial and adaxial meristeles fuse each other at the middle part of the lamina. In *Cheiropleuria* they do not fuse with each other, but are arranged separately in a horizontal. Thus, the stelar system of *Cheiropleuria* is quite unique.

The chief interest is centred on the main veins. In this species, there are 4-6 main veins in the lamina as the result of twice of thrice bifurcations in the petiole, showing no midrib. Such features of leaves are rare and are considered as primitive.

The dimorphic character of leaves is apparently a condition which is not rare among ferns. In discussing *Cheiropleuria*, however, this characteristics is also important. The sterile leaves, which are characterized by the wide, firm leatherly

lamina, are borne on a thin wiry petiole, which resembles especially with *Dipteris*.

The form and the venation of the lamina, providing more than two cusps, resemble closely to those of *Dipteris*, although in *Cheiropleuria*, the one-cusped type is sometimes dominant.

Wagner (1952) distinguished four general types of the foliar dichotomy among the living ferns and put this species, together with *Ctenopteris heteromorpha*, *Grammitis furcata* and *Platynerium*, in the crested blade type. He says that the leaves of juvenile plants resemble to the fertile in the reticulate architecture of veins. But in *Cheiropleuria* adult leaves are pinnate-reticulate in vascular architecture, and juvenile leaves rather resemble to those of the *Dipteris*. The adult fertile leaves are found to be alike to those of most species of the Polypodiaceae. Also, the fact that juvenile leaves are entire, is due to undeveloped of the main veins. A preponderance of main veins over small veins are the later than of this stage. Though, in *Platynerium* the crested blade becomes stable, in *Cheiropleuria* it is not so. Thus, there are some differences between both genera in minute feature of the venation as well as in the degree of the stability of the crestal character.

No peculiarities are found in the structures of the root. The presence of well-developed sclerenchymatous tissue in the cortex resembles to that of the most species of *Equisetum*, *Marattia*, and *Selaginella*, while the structure of the stele resembles to some species of Ophioglossaceae and *Marattia*.

On the other hand, the gametophyte in this species has a certain number of some characteristics which differ from the Polypodiaceae. Recently, Stocky and Atkinson (1954) published a detailed study on the development of the gametophyte and described that this species differed markedly in various features from the higher ferns, but rather resembled to the members of the Gleicheniaceae, Matoniaceae and Dipteridaceae. They recognized the family Cheiropleuriaceae though the characteristics pointed out by them are somewhat different from those by Nakai who proposed this family in 1933 on the basis of the gametophyte as having the endophytic fungi, and archegonia with straight neck. Also the writer rather roughly agrees with Stocky and Atkinson (1954) in the following points, that is, the base is cordate, endophytic fungi are not found, the archegonium is long and large, and the midrib is 8-10 cells thick.

In conclusion, this species shows an abundance of primitive characteristics in the sporophyte as well as in the gametophyte, namely, the typical protostelic stem, non-articulate stipes with two vascular strands at its base, simple scales, glabrous surface of the lamina, and obliquely placed annulus of the sporangium with a stalk composed of four rows of cells. Especially, the two meristemes at the petiolar base, the repeating bifurcations of the veins, various characters of juvenile leaves, and the form of the lamina, as well as particular characters of the gametophyte, are the characteristics which cannot be found in any of other ferns.

The morphological and anatomical characters of *Cheiropleuria* are indeed pri-

mitive and show a few affinity to other ferns. An assortment of such characteristics of sporophyte further indicates that the *Cheiropleuria* should be placed in an independent family. Thus the Cheiropleuriaceae Nakai which was established on the basis of a study on the gametophyte is also recognizable from the results of the present study.

Summary

Anatomical and morphological studies have been carried on the sporophytes, especially on the leaves, of *Cheiropleuria bicuspis* var. *integrifolia* from Hachijo Island. Gametophytes which persist imperfectly on some juvenile plants are also observed. There is an abundance of particular and primitive characteristics, such as, (a) the protostele showing a dorsiventrality in the dominant number of the protoxylem groups on its dorsal side; (b) a marked dimorphism shown as the sterile and fertile leaves; (c) firm and leathery laminas which are frequently bicusped at their tips and are provided with 4-6 parallel veins formed by repeated bifurcations of bundles of *Marattia*-type in the non-articulated and wiry petiole, etc. An assortment of such characteristics of the sporophytes apparently shows an independent systematic position of *Cheiropleuria* among the Leptosporangiate ferns, and, thus, strongly supports the opinion of Nakai, who established an independent family Cheiropleuriaceae on the basis of some particular characteristics of the gametophyte.

Finally the writer wishes to acknowledge his indebtedness to Prof. Y. Ogura and Dr. S. Watari for his kindness of reading the original manuscript. He also thanks to Prof. T. Kondo of Biological Institute of the Shizuoka University for his kindness of giving permission to use his collection. He is also grateful to J. Tsuboi who helped him in collection of the material at Hachijo Island.

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キンボウゲ科の細胞学的研究 I

キンボウゲ属の核型分析

栗 田 正 秀*

Masahide KURITA: Cytological Studies in Ranunculaceae I
The Karyotype Analysis in the Genus *Ranunculus*

1954 年 11 月 25 日受付

緒 言

キンボウゲ属 (*Ranunculus*) の染色体研究はすでに多数の研究者例えば Senjaninova⁷⁾, Sorokin⁸⁾, Langlet^{3,4)}, Larter⁵⁾, 松浦及須藤⁶⁾, Böcher¹⁾, Coonen²⁾, 等によつておこなわれたが、核型に関する詳細な報告はすくない。本属植物は我国に約 30 種野生するが、このうち核型がくわしく分析されたのは松浦及須藤⁶⁾によつて研究された数種にすぎず、他の種においては核型分析が不十分か又は全くおこなわれていない。筆者はさらに多数の種の核型を分析し、本属で知られている基本数 6, 7 及び 8 の相互の関係を解明する目的で研究をおこなっている。

ここで 5 種 3 変種についておこなつた核型研究の結果を報告する。

方 法

材料植物から切りとられた根端の前処理及び固定染色には Tjio & Levan¹⁰⁾ の方法を一部変更した方法がもちいられた。即ち根端を 0.002 M の 8-Oxyquinoline 水溶液に 2~3 時間浸して後、約 10 分間水道水であらつた。次に 60°C の N-HCl で約 15 秒間処理し、ただちにのせガラス上にとり出して醋酸 Orcein 一滴を加え、ふたガラスをかぶせて一層細胞になるようおさえつけた。花粉母細胞の減数分裂は母細胞をなすりつけ法により鉄醋酸 Carmine 液で固定染色して観察した。図はすべて 2600 倍に描画したが、印刷に際し 1170 倍に縮小した。

観 察

1. ウマノアシガタ *Ranunculus japonicus* Thunb. (松山市産); ヒメウマノアシガタ *R. yakushimensis* (Makino) Masam. (鹿児島県屋久島産)

筆者はウマノアシガタの根端細胞で 14 個の染色体を観察した。これら染色体中には不等対はみとめられない。半数染色体組に属する 7 染色体のうち、3 個 (Fig. 1. a~c) は中部着糸点をもっており、うち 1 個 (*Ibid.* c) は他よりやや短い。残り 4 個 (*Ibid.* d~g) は次端部に着糸点をもっているが、その各短腕の長さは 3 個 (*Ibid.* d~f) では染色体の幅より長く、1 個 (*Ibid.* g) ではやや短くて球形を呈しいる。小さい付随体が最後に述べた染色体の短腕に存在する。

Fig. 1. Somatic chromosomes of *R. japonicus*. $\times 1170$

筆者はヒメウマノアシガタの核型と上述のウマノアシガタのそれとの間に差異を認めなかつた。したがつて両種の核型は次の式で示せる。

$$K(2n)=14=2A_1^m+2A_2^m+2B^m+2C_1^{st}+2C_2^{st}+2C_3^{st}+2t \text{ Dst}$$

2. チシマキンボウゲ *R. auricomus* L. (北千島産) Langlet⁴⁾ 及び Böcher¹⁾ は本種の染色体

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数を $n=16$, $2n=32$ と報告しているが、筆者の材料では $2n=28$ と決定した。Fig. 2 にしめすようにこれら 7 染色体は構成員がそれぞれよく似てよいる 4 組に区分することができる。この各組は構成員の数と形態からみてウマノアシガタの半数染色体組に非常によく似ている。したがって染色体形態の立場だけから判断すると本種は 7 を基本数とする同質四倍体とかがえらる。

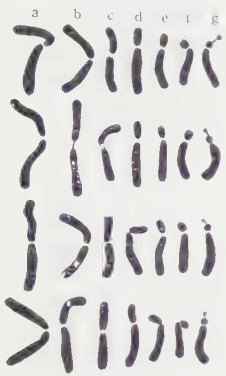


Fig. 2. Somatic chromosomes of *R. auricomus*. $\times 1170$

本種は次の式でしめせる。

$$K(2n)=28=4A_1^m+4A_2^m+4B^m+4C_1^{st}+4C_2^{st}+4C_3^{st}+4D^{st}$$

3. キツネノボタン *R. ternatus* Thunb. var. *glaber* H. Boiss. (松山市産); ヤマキツネノボタン *R. ternatus* Thunb. var. *quelpaertensis* (Léveillé) Ohwi (愛媛県大野原産)

筆者は両変種の染色体数をそれぞれ $2n=16$ と決定し、また両者の核型間に差異をみとめなかつた。

Fig. 3 はヤマキツネノボタンの染色体をしめているが、同図であきらかなように 16 個の染色体中には不等対はない。半数染色体組に属する 8 染色体のうち、3 個 (*Ibid.* a~c) は V 字形、5 個 (*Ibid.* d~h) は J 字形である。V 字形染色体は順次わずかに長さに差があり a-染色体の着糸点は中部に、b 及び c のそれは中部に極めて近

い次中部にある。J 字形染色体のうち d~f の 3 個ではその各制対は染色体の幅より長く、g 染色体では短い。最後の h 染色体では Fig. 4 に模式図に示したように、一端が尖っており多くの場合付随体状の小体を先端にもつた糸状物が突出して (*Ibid.* b, c), Fig. 4. a の如く観察される場合は極めて稀にみつた。この染色体の主体をなす部分うち上記の尖った端と凡い他の端との間には着糸点とおもわれるところは認められない。尖端ちかくを注意して観察すると図の b 及び c に示すように尖端に接してか、又はやや離れて糸状物上にうすく染まる物質が認められる。この物質は微小となつた短腕なのか又は Tjio & Levan¹⁰⁾ の云う centromeric body の集まつたものなのかの決定は困難であるが、その染色の度合や輪郭の不規則な点から筆者はその物質をおそらく後者であろうと推定する。したがって糸状物の先端にある濃染する付随体状小体はこの染色体の微小な短腕に相当するものと考ええる。即ち h 染色体では着糸点のあるくびれの部分が仁形成に関係しているため、そこが引きのばされ、したがって微小な短腕が付随体状となるのではなかろうか。そこでこの h 染色体を tDst とせず Dst であらわせば本種の核型は次のように示せる。

$$K(2n)=16=2A_1^m+2A_2^{sm}+2A_3^{sm}+2B_1^{st}+2B_2^{st}+2B_3^{st}+2C^{st}+2D^{st}$$

4. ケキツネノボタン *R. cantoniensis* DC. (松山市産)



Fig. 5. Somatic chromosomes of *R. cantoniensis*. $\times 1170$

本種の染色体数は $2n=32$ である。これら 32 個の染色体は構成員がそれぞれよく似た 4 組にわけることができる (Fig. 5)。各組に属する 8 染色体は前述のヤマキツネノボタンの半数染色体組の各染色体にそれぞれよく似ている。したがって本種は染色体形態上からみて 8 を基本数とする同質四倍体であろう。

核型は次の式で示せる。

$$K(2n)=32=4A_1^m+4A_2^{sm}+4A_3^{sm}+4B_1^{st}+4B_2^{st}+4B_3^{st}+4C^{st}+4D^{st}$$

Fig. 3~4. 3, Somatic chromosomes of *R. ternatus* var. *quelpaertensis*. $\times 1170$. 4, Diagrams of the constricted part of h-chromosome in fig. 3.

5. タガラシ *R. scleratus* L. (松山市産)

Langlet⁴⁾と同様に筆者も本種の染色体数を $2n=32$ と決定した。花粉母細胞の減数第一分裂で大多数の染色体は二価染色体となり、時に 16II の対合も認められた。この対合状況及び体細胞染色体の形態から 32 個の染色体は Fig. 6 に示すように構成員がそれぞれに相似た二つの半数染色体組



Fig. 6. Somatic chromosomes of *R. scleratus*. $\times 1170$

に分けることができる。各組の 16 染色体のうち、8 個は順次わずかに長さに差がある V 字形染色体で何れも中部着糸点をもっており、最大染色体の $1/2$ 以下の長さのものが 2 個ある。残り 8 個は J 字形で前者の場合と同様、最大染色体の $1/2$ 以下の長さのものが 2 個ある。Coonen²⁾ は本種で大きい付随体をもった染色体 1 対を観察しているが筆者はかような染色体を認めなかつた。

本種の核型は次のように示せる。

$$K(2n)=32=12A^m+4B^m+12C^{st}+4D^{st}$$

6. イトキンボウゲ *R. reptans* L. var. *flagellifolius* (Nakai) Ohwi (日光市産)

この変種の染色体数は $2n=32$ である。これら染色体のうち、16 個は V 字形で大きい 12 個



Fig. 7. Somatic chromosomes of *R. reptans* var. *flagellifolius*. $\times 1170$

長さに差がみられる (Fig. 7)。

核型は次の式で示せる。

$$K(2n)=32=12A^m+4B^{sm}+16C^{st}$$

タガラシとイトキンボウゲの染色体はここで報告された他植物のそれより一般に小さく、とくに

各植物の最小染色体についてはこの差異が顕著である。ともに $2n=32$ の染色体数をもっているケキツネノボタン、タガラシ及びイトキンボウゲにおいて各最小染色体の長さを測定したがその結果は次のようである。即ちケキツネノボタンのそれを 100 とすればタガラシのは 36、イトキンボウゲのは 40 である。

考 察

ウマノアシガタ、ヒメウマノアシガタ及びチシマキンボウゲは 7 を、キツネノボタン、ヤマキツネノボタン及びケキツネノボタンは 8 を基本数とする系列に属する。前者の基本核型と後者のそれとの間には一二わずかの相違もあるが最も顕著な差異は次のようである。即ち Fig. 1 と Fig. 3 によつて述べると Fig. 1 の **g** 染色体は付随体をもつことと Fig. 3 のそれと異り、Fig. 3 の **h** 染色体と等しい染色体は Fig. 1 には見出されない。Coonen²⁾ は基本数 7 は基本数 8 から染色体消失によつて由来したと推定している。いま上述の顕著な差異のみについてみるに、Fig. 3 から **h** 染色体を除去しても残りの染色体は Fig. 1 のそれとは付随体の有無によつて一致しない。したがつて 1 染色体の完全消失によつては両基本核型の関係は説明できない。しかし **h** 染色体がくびれの部分で切断し、その短腕が仁形成能力をもなつて **g** 染色体の短腕に転座し、**h** の長腕は消失したとすれば説明し得るようにおもえる。

タガラシ及びイトキンボウゲは染色体数からみると基本数 8 の系列に属するが、染色体の形と大きさから見る時、同系列に属するキツネノボタン、ヤマキツネノボタン及びケキツネノボタンとは非常に異つてゐる。したがつて 8 を基本数とする系列の中に 2 系統があるといえる。

この研究中親切な御指導をたまわつた下斗米教授に對し厚く御礼申上げる。また東京大学付属植物園日光分園の久保田秀夫氏から材料の一部蒐集のため多大の御援助をいただいた。ここに記して感謝の意をあらわす。

Summary

1. The karyotypes of five species and three varieties in *Ranunculus* are determined as follows:

<i>R. japonicus</i>	$K(2n)=14=2A_1^m+2A_2^m+2B^m+2C_1^{st}+2C_2^{st}+2C_3^{st}+2tD^{st}$
<i>R. yakushimensis</i>	$K(2n)=14=2A_1^m+2A_2^m+2B^m+2C_1^{st}+2C_2^{st}+2C_3^{st}+2tD^{st}$
<i>R. auricomus</i>	$K(2n)=28=4A_1^m+4A_2^m+4B+4C_1^{st}+4C_2^{st}+4C_3^{st}+4tD^{st}$
<i>R. ternatus</i> var. <i>glaber</i>	$K(2n)=16=2A_1^m+2A_2^{sm}+2A_3^{sm}+2B_1^{st}$ $+2B_2^{st}+2B_3^{st}+2C^{st}+2D^{st}$
<i>R. ternatus</i> var. <i>quelapaertensis</i>	$K(2n)=16=2A_1^m+2A_2^{sm}+2A_3^{sm}+2B_1^{st}$ $+2B_2^{st}+2B_3^{st}+2C^{st}+2D^{st}$
<i>R. cantoniensis</i>	$K(2n)=32=4A_1^m+4A_2^{sm}+4A_3^{sm}+4B_1^{st}$ $+4B_2^{st}+4B_3^{st}+4C^{st}+4D^{st}$
<i>R. sceleratus</i>	$K(2n)=32=12A^m+4B^m+12C^{st}+4D^{st}$
<i>R. reptans</i> var. <i>flagellifolius</i>	$K(2n)=32=12A^m+4B^{sm}+16C^{st}$

2. Comparing the 7-basic complement in fig. 1 with the 8-basic one in fig. 3, we find the former to differ markedly from the latter in the absence of a chromosome corresponding to **h** in fig. 3 and the presence of a satellite **g** in fig. 1.

3. From the karyotype analysis, the race with the basic number 8 is divided into two groups. The first includes *R. cantoniensis* and the two varieties of *R. ternatus*, and the second *R. sceleratus* and *R. reptans* var. *flagellifolius*.

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ノコンギク属植物の核分析 III*

藤 原 悠 紀 雄

Yukio HUZIWARA: Karyotype Analysis in *Aster* III**

1954 年 12 月 27 日受付

筆者^{4, 5} は前 2 報において *Aster* 属 11 種, 4 亜種, および 1 変種について核型を明かにした。引続き 3 種, 2 亜種および 2 変種について核型分析を行つたのでここに報告する。用いた 7 植物のうち 1 種および 2 変種は外部形態において 2 つの種または亜種の間形を示し分類学的に天然の雑種と見られるものである。

材料および方法

核型観察の方法は前回と同様であつて用いた材料は次の通りである。

Euaster 節

Aster fastigiatus Fischer ヒメシオン

A. tenuipes Makino クルマギク

A. ageratoides Turcz subsp. *leiophyllus* Kitamura var. (*A. ageratoides* Turcz subsp. *leiophyllus* Kitamura × *A. ageratoides* Turcz subsp. *ovatus* Kitamura)

A. ageratoides subsp. *amplexifolius* Kitamura var. (*A. ageratoides* Turcz subsp. *amplexifolius* Kitamura × *A. ageratoides* Turcz subsp. *leiophyllus* Kitamura)

A. ageratoides Turcz subsp. *tubulosus* Kitamura チョクザキヨメナ

A. ageratoides Turcz subsp. *ripensis* Kitamura タニガワコンギク

Teretiachenium 節

A. Sekimotoi Makino (*A. scaber* Thunb. ×

A. rugulosus Maxim.) ナガバシラヤマギク

結 果

1. ヒメシオン *Aster fastigiatus* Fischer $2n=18$ 愛知船着産 (Fig. 1, Table I)

体細胞染色体 18 個は大きさの順に 9 対に排列することができ形の上から 7 種類に区別される。着糸点は 2 対 (3, 4; 17, 18) が median で, 他はすべて submedian である。最大の染色体 1 対 (1, 2) は L²E 染色体で短腕に二次狭窄をもち長さ 7.8 μ あり, 最小の対は長さ 4.8 μ である。

核型は次の式で表わされる。

$$K(2n)=18=2csA^{sm}+2B^m+2C_1^{sm}+2C_2^{sm}+2C_2^{sm}+2D^{sm}+6E^{sm}+2F^m$$

Table I. Measurements on somatic chromosomes in *A. fastigiatus*

Chromosomes	Length in μ	Relative length
1, 2	7.8=4.2+2.4+1.2	100
3, 4	7.2=3.6+3.6	92
5, 6	6.6=4.2+2.4	85
7, 8	6.6=3.6+3.0	85
9, 10	6.0=3.6+2.4	77
11~16	5.4=3.0+2.4	69
17, 18	4.8=2.4+2.4	62

2. クルマギク *A. tenuipes* Makino $2n=18$ 和歌山那智山産 (Fig. 2, Table II)

本種は和歌山県南部地方にのみ見られる稀産種であつて, *A. ageratoides* とは外部形態において類似するが核型においてはやゝ趣を異にする。体細胞染色体 18 個は大きさの順に 6 対に排列することができ, 形の上から 6 種類に区別できる。着糸点は 1 対 (3, 4) が subterminal 1 対 (5, 6) が

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** Contributions from the Biological Institute, Kôbe University No. 24

median, 残る 7 対はすべて Submedian である。最大の染色体 1 対 (1, 2) は L₂E 染色体で短腕に二次狭窄を有し長さ 8.4 μ あり, 最小の染色体は長さ 5.4 μ である。

核型は次の式で表わされる。

$$K(2n)=18=2csA^{sm}+2B^{st}+2C^m+2D^{sm}+8E^{sm}+2F^{sm}$$

Table II. Measurements on somatic chromosomes in *A. tenuipes*

Chromosome	Length in μ	Relative length
1, 2	8.4=4.8+2.4+1.2	100
3, 4	7.8=5.4+2.4	93
5, 6	7.2=3.6+3.6	86
7, 8	6.6=4.2+2.4	79
9~16	6.0=3.6+2.4	71
17, 18	5.4=3.0+2.4	64

3. *A. ageratoides* Turcz subsp. *leiophyllus* Kitamura var. (*A. ageratoides* Turcz subsp. *leiophyllus* Kitamura \times *A. ageratoides* Turcz subsp. *ovatus* Kitamura) $2n=36$ 舞鶴産 (Fig. 3, Table III)

本植物は兵庫, 京都, 福井各府県の日本海岸に生育し外部形態においてシロヨメナとノコンヤクとの中間形を示す。核型はノコンギクに類似し, 大小 2 対の L₂E 染色体をもち, 着糸点は 1 対

(25, 26) のみ median で他はすべて submedian である。最大の染色体 1 対 (1, 2) は長さ 8.4 μ あり, 第 2 対 (3, 4) は第 1 対よりわずかに短いがいずれも短腕に二次狭窄をもっている。最小の 1 対は長さ 5.4 μ である。

核型は次の式で表わされる。

$$K(2n)=36=2csA^{sm}+2csB^{sm}+2C_1^{sm}+2C_2^{sm}+4D_1^{sm}+2D_2^{sm}+10E_1^{sm}+2E_2^m+10F^{sm}$$

4. ヤマシロギク *A. ageratoides* Turcz subsp. *amplexifolius* Kitamura var. (*A. ageratoides* Turcz subsp. *amplexifolius* Kitamura \times *A. ageratoides* Turcz subsp. *leiophyllus* Kitamura) $2n=36$ 神戸六甲山産 (Fig. 4, Table IV)

本植物は近畿および中国の山地に広く分布し, 外部形態においてイナカギクとシロヨメナとの中間形を示す。体細胞染色体 36 個は形と大きさから 10 種類に区別される。着糸点は 6 対 (1, 2; 5, 6; 17, 18; 29, 30; 31, 32; 33, 34) が median で残る 12 対はすべて submedian である。最大の染色体 1 対は L₂E 染色体で一方の腕に二次狭窄をもち長さ 8.4 μ である。最小の染色体は長さ 4.2 μ である。

核型は次の式で表わされる。

$$K(2n)=36=2csA^m+2B_1^{sm}+2B_2^m+6C^{sm}+4D_1^{sm}+2D_2^m+6E^{sm}+4F_1^{sm}+6F_2^{sm}+2G^{sm}$$

Table IV. Measurements on somatic chromosomes in *A. ageratoides* subsp. *amplexifolius* var. (*A. ageratoides* subsp. *amplexifolius* \times *A. ageratoides* subsp. *leiophyllus*)

Chromosomes	Length in μ	Relative length
1, 2	8.4=4.2+3.0+1.2	100
3, 4	7.2=4.2+3.0	86
5, 6	7.2=3.6+3.6	86
7~12	6.6=3.6+3.0	79
13~16	6.0=3.6+2.4	71
17, 18	6.0=3.0+3.0	71
19~24	5.4=3.0+2.4	64
25~28	4.8=3.0+1.8	57
29~34	4.8=2.4+2.4	57
35, 36	4.2=2.4+1.8	50

Table III. Measurements on somatic chromosomes in *A. ageratoides* subsp. *leiophyllus* var. (*A. ageratoides* subsp. *leiophyllus* \times *A. ageratoides* subsp. *ovatus*)

Chromosomes	Length in μ	Relative length
1, 2	8.4=4.8+2.4+1.2	100
3, 4	7.8=4.2+2.4+1.2	93
5, 6	7.2=4.8+2.4	86
7, 8	7.2=4.2+3.0	86
9~12	6.6=4.2+2.4	79
13, 14	6.6=3.6+3.0	79
15~24	6.0=3.6+2.4	71
25, 26	6.0=3.0+3.0	71
27~36	5.4=3.0+2.4	64

5. チョクザヨメナ *A. ageratoides* Turcz subsp.

tubulosus Kitamura $2n=36$ 栽培 (Fig. 5, Table V)

体細胞染色体36個は形と大きさから11種類に区別される。着糸点は2対(27, 28; 29, 30)が subterminal, 4対(17, 18; 19, 20; 31, 32; 33, 34)が Median で残る12対はすべて submedian である。最大の1対(1, 2)は L^2E 染色体で短腕に二次狭窄をもち長さ 7.8μ あり, 最小の染色体は長さ 1.8μ である。

核型は次の式で表わされる。

$$K(2n)=36=2cs2A^{sm}+2B_1^{sm}+2B_2^{sm}+4C^{sm}+6D^{sm}+2E^m+2F^m+6G^{sm}+4H_1^{st}+4H_2^m+2I^{sm}$$

6. タニガワコンギク *A. ageratoides* Turcz

Table V. Measurements on somatic chromosomes in *A. ageratoides* subsp. *tubulosus*

Chromosomes	Length in μ	Relative length
1, 2	$7.8=4.2+2.4+1.2$	100
3, 4	$6.6=4.2+2.4$	85
5, 6	$6.6=3.6+3.0$	85
7~10	$6.0=3.6+2.4$	77
11~16	$5.4=3.0+2.4$	69
17, 18	$4.8=2.4+2.4$	62
19, 20	$3.6=1.8+1.8$	46
21~26	$3.0=1.8+1.2$	38
27~30	$2.4=1.8+0.6$	31
31~34	$2.4=1.2+1.2$	31
35, 36	$1.8=1.2+0.6$	23

Table VI. Measurements on somatic chromosomes in *A. ageratoides* subsp. *ripensis*

Chromosomes	Length in μ	Relative length
1, 2	$7.2=4.2+1.8+1.2$	100
3~6	$6.0=3.6+2.4$	83
7~10	$5.4=3.0+2.4$	75
11, 12	$4.8=3.0+1.8$	67
13~16	$4.8=2.4+2.4$	67
17, 18	$4.2=2.4+1.8$	58
19~30	$3.0=1.8+1.2$	42
31~36	$2.4=1.2+1.2$	33

subsp. *ripensis* Kitamura $2n=36$ 広島南原産 (Fig. 6, Table VI)

体細胞染色体26個は形と大きさから8種類に区別される。着糸点は5対(13, 14; 15, 16; 31, 32; 33, 34; 35, 36)が median で他はすべて submedian である。最大の1対(1, 2)は L^2E 染色体で短腕に二次狭窄をもち長さ 7.2μ あり, 最小の染色体は長さ 2.4μ である。

核型は次の式で表わされる。

$$K(2n)=36=2csA^{sm}+4B^{sm}+4C^{sm}+2D_1^{6m}+4D_2^m+2E^{sm}+12F^{sm}+6G^m$$

7. ナガバシラヤマギク *A. Sekimotoi* Makino $2n=18$ 京都甘南備山産 (Fig. 7, Table VII)

本種は栃木, 滋賀, 京都の各府県に産し外部形態においてシラヤマギク *A. scaber* とサワシロギク *A. rugulosus* との間形を示し両種の雑種と考えられる。体細胞染色体18個は大きさの順に9対に排列することができ, 形の上から7種類に区別される。着糸点は3対(9, 10; 11, 12; 13, 14)が median で, 残る6対は submedian である。最大の染色体1対(1, 2)は L^2E 染色体で短腕に二次狭窄をもち長さ 10.2μ あり, 最小の染色体も短腕に二次狭窄をもち長さ 5.4μ である。

核型はサワシロギクと類似しつぎの式で表わされる。

$$K(2n)=18=2csA^{sm}+2B_1^{sm}+2B_2^{sm}+2C^{sm}+6D^m+2E^{sm}+2csF^{sm}$$

Table VII. Measurements on somatic chromosomes in *A. Sekimotoi*

Chromosomes	Length in μ	Relative length
1, 2	$10.2=5.4+2.4+2.4$	100
3, 4	$8.4=5.4+3.0$	82
5, 6	$8.4=4.8+3.6$	82
7, 8	$7.8=4.8+3.0$	76
9~14	$7.2=3.6+3.6$	71
15, 16	$6.6=3.6+3.0$	65
17, 18	$5.4=3.0+1.2+1.2$	53

考察と結論

日本産 *Aster* のうち *Euaster*, *Teretiachenium* 両節のものは例外なく L^2E 染色体を有すること

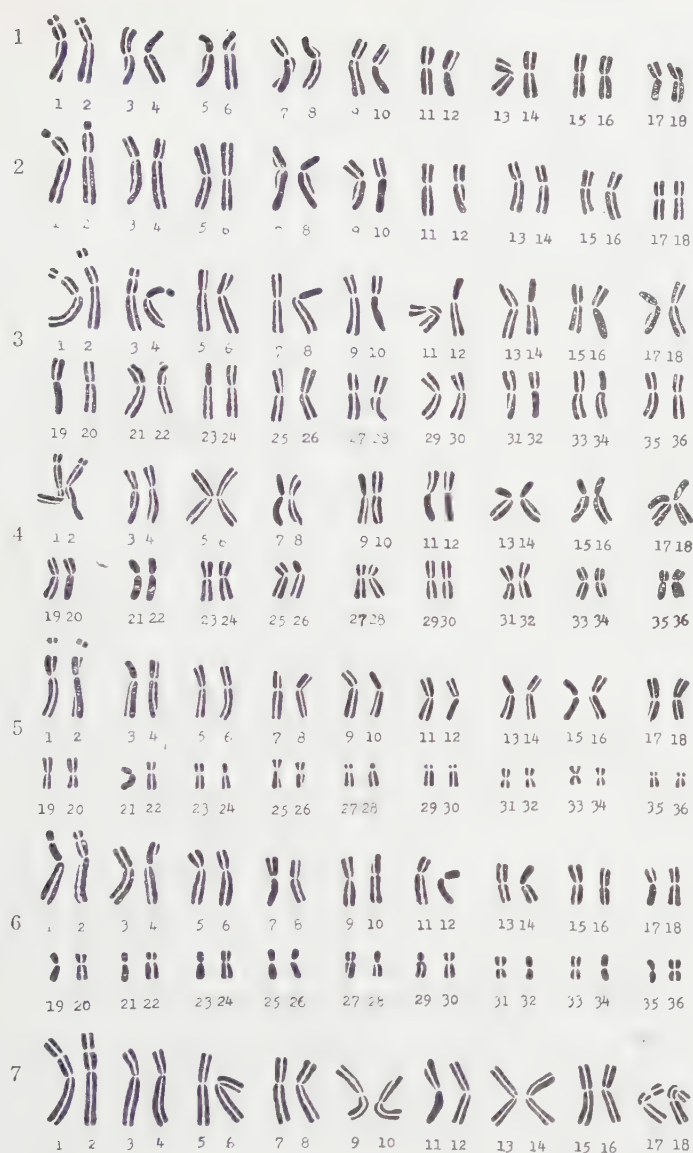
Figs. 1~7. Somatic chromosomes $\times 1500$

Fig. 1. *Aster fastigiatus*. Fig. 2. *A. tenuipes*. Fig. 3. *A. ageratoides* subsp. *leiophyllus* var. (*A. ageratoides* subsp. *leiophyllus* \times *A. ageratoides* subsp. *ovatus*). Fig. 4. *A. ageratoides* subsp. *amplexifolius* var. (*A. ageratoides* subsp. *amplexifolius* \times *A. ageratoides* subsp. *leiophyllus*). Fig. 5. *A. ageratoides* subsp. *tubulosus*. Fig. 6. *A. ageratoides* subsp. *ripensis*. Fig. 7. *A. Sekimotoi*. (*A. scaber* \times *A. rugulosus*).

Summary

1. The karyotypes of 3 species, 2 subspecies and 2 varieties in *Aster* are reported.
2. The karyotype formulae are as follows:

は今回の報告においても確めることができた。ヒメシオン、クルマユク、ナガバシラヤマギクなどの2倍種および4倍種のうちイナカギクとシロヨメナとの中間形を示すもの、チョクザキヨメナ、タニガワコンギクなどにおいてはそれぞれ1対ずつあり、シロヨメナとノコンギクとの中間形を示す植物においては2対ある。しかしこの2対の染色体は互に長さを異にするから核型から見てこれらの4倍種はいずれも異質4倍種といえる。Aversl. (2, 3) は北アメリカ産 *Aster* のうち *Euaster* 節, *Heterophylli* 系に属する4倍種が同質4倍種またはそれに近い部分的異質4倍種であることを報告したが日本産 *Aster* の倍数種が核型から見てすべて異質倍数種であることは興味がある。分類学上天然における雑種と見られる1種および2変種はいずれもかなり広い分布区域をもち核型の上では雑種性を示さずむしろ安定した2倍種または4倍種の核型を有するところから独立の種または変種と見做してよいものと思われる。

御懇切なる御指導を賜っている広島大学下斗米教授に感謝の意を表し、分類学上の御教示を賜わり貴重なる材料植物を惠与下さった京都大学北村教授に御礼申上げる。

Aster fastigiatus

$$K(2n)=18=2^{cs}A^{sm}+2B^m+2C_1^{sm}+2C_2^{sm}+2D^{sm}+6E^{sm}+2F^{sm}$$

A. tenuipes

$$K(2n)=18=2^{cs}A^{sm}+2B^{cs}+2C^m+2D^{sm}+8E^{sm}+2F^{sm}$$

A. ageratoides subsp. *leiophyllus* var. (*A. ageratoides* subsp. *leiophyllus* × *A. ageratoides* subsp. *ouatus*)

$$K(2n)=36=2^{cs}A^{sm}+2^{cs}B^{sm}+2C_1^{sm}+2C_2^{sm}+4D_1^{sm}+2D_2^{sm}+10E_1^{sm}+2E_2^m+10F^{sm}$$

A. ageratoides subsp. *amplexifolius* var. (*A. ageratoides* subsp. *amplexifolius* × *A. ageratoides* subsp. *leiophyllus*)

$$K(2n)=36=2^{cs}A^m+2B_1^{sm}+2B_2^m+6C^{sm}+4D_1^{sm}+2D_2^m+6E^{sm}+4F_1^{sm}+6F_2^m+2G^{sm}$$

A. ageratoides subsp. *tubulosus*

$$K(2n)=36=2^{cs}A^{sm}+2B_1^{sm}+2B_2^{sm}+4C^{sm}+6D^{sm}+2E^m+2F^m+6G^{sm}+4H_1^{st}+4H_2^m+2I^{sm}$$

A. ageratoides subsp. *ripensis*

$$K(2n)=36=2^{cs}A^{sm}+4B^{sm}+4C^{sm}+2D_1^{sm}+4D_2^m+2E^{sm}+12F^{sm}+6G^m$$

A. Sekimotoi (*A. scaber* × *A. rugulosus*)

$$K(2n)=18=3^{cs}A^{sm}+2B_1^{sm}+2B_2^{sm}+2C^{sm}+6D^m+2E^{sm}+2^{cs}F^{sm}$$

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抄 録

アマノリ類の生活史

[Drew, K. M.: Life-history of *Porphyra* Nature 173: 1243 (1954)]

アマノリ属における *Conchocelis* phase のもの (介殻上におちた果胞子が発芽後殻中に穿孔して出来た糸状体) の存在は Drew (1949) の最初の報告に次ぐ黒木の日本産 4 種のものの観察で確認され、同属の生活史中で共通のであるらしい。其の形態も多くの種のものでよく似ている。然しウシケノリ属では少し違う。*Conchocelis* phase の体上には生殖胞子が多数作られるが、芝等がどう發育して行くのが生活史上次の問題である。黒木は「芝等はすべてがノリ体になり、再び介殻中に穿孔する事はない」と云い、*P. umbricaris* J. Ag. Prox. (チシマクロノリ?) も同様だとする。若しそれがほんとなら、最初に自分がやつた *P. umbilicalis* v. *laciniata* (歐洲産) の場合と違う処があり、アマノリ属でも種が違えば生活史が之と異なる

のか、或いは同一種でも常に同一過程をたどるとは限らないと云うことになる。と云うのは、*P. umbilicalis* v. *laciniata* では「小型ノリ」(恐らく果胞子からすぐノリ体になつた物) も確かに見られるし、また *Conchocelis* の穿孔した介殻と未だ穿入のない新しい介殻とを一緒に入れておくと、くつつけておいても離しておいても、後者の介殻に *Conchocelis* が伝染した。同様な事がウシケノリでも見られた。ここで如何して伝染が行われたかを明らかにせねばならぬ。vegetative 枝によるのか胞子によるのか? 又は黒木が報ずる如く胞子に 2 種あるのか?

とに角アマノリ属の生活史についてはなお多くの問題が残されている。

() 内抄録者註。

(新崎盛敏)

本 会 記 事

会 員 移 動

新 入 会 (1,2 月)

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 稲葉通一、井上正道、石黒道也 以上 25 名 広
 島大理植

住 所 変 更

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 藤田哲夫 広島大理植
 友岡浩 杉並区永福町 323

会 員 名 簿 正 誤

6 頁右 —11 人目 *野口つた
 8 右 6 大島宇内
 11 右 9 奈良県天理市樺本町樺本
 14 右 —12 小谷信矢
 15 左 —11 外山三郎 宮崎大学農生物
 (を加える, 同名同姓)
 15 右 —1 Bower, F. O. をけずる(死亡)

本会名誉会員 G. Tischler 氏(ドイツ, キール大学教授)
 は本年 1 月 6 日 77 才の高齢で死去されました。
 ここに報告し謹んで哀悼の意を表します。

日 本 植 物 学 会

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Habitat Segregation in *Aster subulatus* and Three Species of *Erigeron* Due to Soil Moisture*

by Akira MIYAWAKI**

宮脇 昭： 土壌水分によるハハキギクとアレチノギク属植物のすみわけ

Received January 26, 1955

Introduction

As a means of ecological analysis of the structure of weed communities, the concept of habitat segregation becomes the most important problem to be solved.

The phenomenon of habitat segregation of weeds is divided into two groups based on their relationship between the life form and the growing field of plants. The one is the homogenous habitat segregation and the other is the heterogenous habitat segregation. The former is that in which the same species occurs in different life forms and different habitats, while the latter is that in which some different species occur in different habitats and, in many cases in, different life forms³⁾.

The purpose of the present paper is to investigate ecologically and morphologically a heterogenous habitat segregation between *Aster subulatus* Michx., and the three species of *Erigeron* (*E. sumatrensis* Retz., *E. canadensis* L. and *E. bonariensis* L.), both of which belong to the same life form and growing period, and also to investigate a homogenous habitat segregation in *A. subulatus*.

The author wishes to express his gratitude to Professor Y. Horikawa of the Hiroshima University for suggesting this investigation as well as for constant guidance throughout the course of the work. To Professor Y. Ogura of the Tokyo University, the author is indebted for much valuable advices on the morphological and anatomical work. Further the author owes thanks to Dr. S. Watari of the Tokyo University, Professor M. Kitagawa of the Yokohama National University, Assist. Professor H. Suzuki and Lecturer H. Andô of the Hiroshima University for their helps and valuable suggestions. Thanks are also due to Professor H. Yagi of the Agricultural Institute of the Yokohama National University for all possible help in providing room and apparatus in the time of the soil analyses.

Area and Method of Study

Field surveys were carried out at the growing habitats of *Aster subulatus* and

*1) Read on the 18th annual meeting of the Botanical Society of Japan, October, 1953 at Kanazawa University, Kanazawa City. 2) Supported by a Grant in Aid for Development Scientific Research from the Ministry of Education in 1953.

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Erigeron spp. on the wayside, ditch-wall and vacant ground in Sendamachi, Hiroshima city in the summer of 1951, '52 and '53.

1). Study of vegetation: Analyses of plant communities of *A. subulatus* and *Erigeron* spp. on road-sides and ditch-walls were made by the systematic sampling by belt transects of 0.2-1.0 m in width or quadrats of 1.0 m². Ecological features of the communities were expressed by the floristic list, coverage and frequency of the occurring plants.

2). Examination of the bed soil of communities: The water content of bed soil at the road-side communities and the pure communities was considered with respect to the soil sample of 20 g and measured after the dryness at 110°C° for 20 hours. For the pure communities, the chemical components of bed soil were determined, too. In this determination, total nitrogen and humus were measured by the Kjeldahl's method and the Turin's method respectively, and in the other tests of soil was made use of the Prof. Yagi's soil tester.

3). Morphological study of subterraneous parts of *A. subulatus*: The subterraneous parts of *A. subulatus* in its pure communities and in the *Erigeron* communities were studied morphologically and anatomically for the comparison in both communities.

Data and Discussion

I. Heterogenous habitat segregation between *A. subulatus* and three species of *Erigeron*:

1) Roadside communities and their soil moisture

For the vegetation developed in and by a green belt zone of road in front of the Western gate of the Hiroshima University, investigations were carried out in September, 1951. To facilitate the investigation, the vegetation was divided into 7 transversal zones A~E from a physiognomical view point (Fig. 1, Tab. I). At the A zone (0.3 m in width) nearer to the side of the high speed vehicle road-way, *A. subulatus* is most dominant in coverage and frequency. But the opposite B' zone is dominated by *E. sumatrensis* and is very few in *A. subulatus*. On the C, C', and D

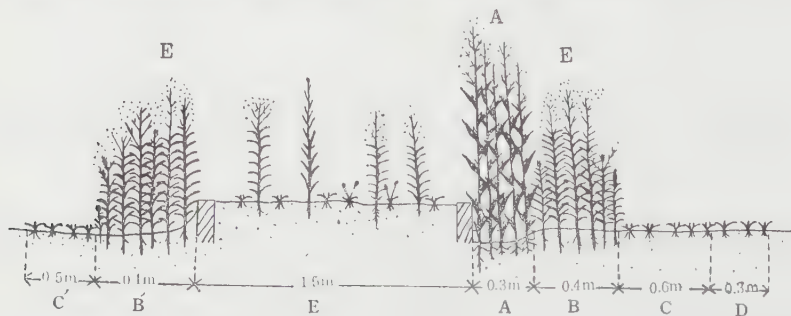


Fig. 1. Diagrammatic beisect through a green belt zone vegetation
A, *Aster subulatus*; E, *Erigeron* spp.

zones which are adjoined to the bare land and influenced by the traffic of vehicles, *Digitaria ascendens* Henr., *D. violascens* Link. and *Eleusine indica* Gaerten are abundant³⁾.

As to the topography of the habitat, the road-surface is generally flat, but the high speed vehicle way is slightly convex and is a little depressed at the adjoined to the green zones. Namely, the part touching the green belt zone exhibits a slope of 5° to 10° at the place of 0.3-0.4 m apart from the paving-stone (near the boundary-line between **A** and **B** zones) and is favoured with the richness of soil moisture and rubbish. On the other hand, the opposite **B'** zone is subjected to the accumulation of soil and slightly raised. In consequence, rain-water on the **B'** zone runs down to the opposite edge of the road:

Table I. Structure of the vegetation in and by a green belt zone in the 113 road-way at Sendamachi, Hiroshima city (showing coverage and frequency obtained from the 10 quadrats with 1 m² area in each zone)

Division of Vegetation Species	A		B		B'		C		C'		D		E	
	C. d.	Fq.	C. d.	Fq.	C. d.	Fq.	C. d.	Fq.	C. d.	Fq.	C. d.	Fq.	C. d.	Fq.
<i>Aster subulatus</i>	5.0	100	0.7	50	—	—	—	—	—	—	—	—	—	—
<i>Erigeron sumatrensis</i>	0.7	40	4.1	100	4.6	100	—	—	—	—	—	—	1.0	30
<i>E. canadensis</i>	0.7	50	3.7	100	2.1	90	0.2	20	—	—	—	—	2.6	70
<i>E. bonariensis</i>	0.2	10	3.2	90	2.0	80	0.2	10	—	—	—	—	1.2	40
<i>Cynodon Dactylon</i>	1.3	30	1.0	20	—	—	1.1	30	—	—	0.6	20	—	—
<i>Setaria viridis</i>	0.2	10	0.5	30	0.1	10	0.3	20	—	—	—	—	1.0	10
<i>Euphorbia sapina</i>	0.2	10	—	—	0.2	10	—	—	—	—	—	—	—	—
<i>Polygonum longisetum</i>	0.1	10	—	—	—	—	—	—	—	—	—	—	—	—
<i>Kummerovia striata</i>	—	—	0.1	10	—	—	—	—	—	—	—	—	—	—
<i>Cyperus rotundus</i>	—	—	0.3	10	—	—	—	—	0.3	10	—	—	—	—
<i>Digitaria ascendens</i>	—	—	0.5	40	0.2	10	1.2	30	—	—	0.2	20	1.7	70
<i>D. violascens</i>	—	—	—	—	2.0	80	4.6	100	0.9	50	0.8	50	4.2	100
<i>Eleusine indica</i>	—	—	—	—	0.2	20	1.2	70	4.9	100	4.6	100	—	—
<i>Eragrostis multicaulis</i>	—	—	—	—	—	—	—	—	—	—	0.1	10	—	—

The results of the determination of soil water content were shown in Tab. II. From this table it is apparent that the soil water content for the sample on the 8th of September*, which is just before a day of rain-fall, is far smaller in every zone than that on the 11th of Sep., which is two days after the rain-fall day. But in both cases, there can be seen a remarkable difference between *E. sumatrensis*-zone (**B**, **B'**) and *A. subulatus*-zone (**A**), the latter of which has the highest water content.

2) Analysis of ditch wall community

In Sendamachi of Hiroshima city, there is a ditch which is about 5 m in width and 2.6 m in depth. The water level of the ditch is always changeable and shows a conspicuous rise even when it encounters a very small rain-fall. There grows a

* While the investigation was going on in 1951, the rainfall was very little from the 17th July to the 10th September.

Table II. Soil water content of each zone in and by a green belt zone showing the mean value of three samples in each zone, and the depth of soil sample portion 10 cm

Division of vegetation	Soil sampling time						
	A	B	B'	C	C'	D	E zone
Just before rain-fall the 9th Sep. '51	7.5	5.9	5.4	4.5	4.4	4.5	3.4 %
Soon after rain-fall the 11th Sep. '51	17.7	15.1	14.6	11.9	13.1	13.9	8.5

dense community of *A. subulatus* and *Erigeron* spp. on the wall. It is observed at a glance that *A. subulatus* grows near the water's edge and *Erigeron* spp. becomes more frequent toward higher part of the wall. To analyse the community 22 belt transects, each of which is 0.2m in width, were set with every 1 m interval at right angle to the stream. Furthermore, quadrats of 0.2 m² were set on each transect and the individual number was carefully investigated with respect to each species. The results are shown in Figs. 2~4. The figures clearly show the heterogenous habitat segregation between *A. subulatus* and *Erigeron* spp. The communities of the respective genus are separated at the place of 1.8~2.0 m high above the water level.

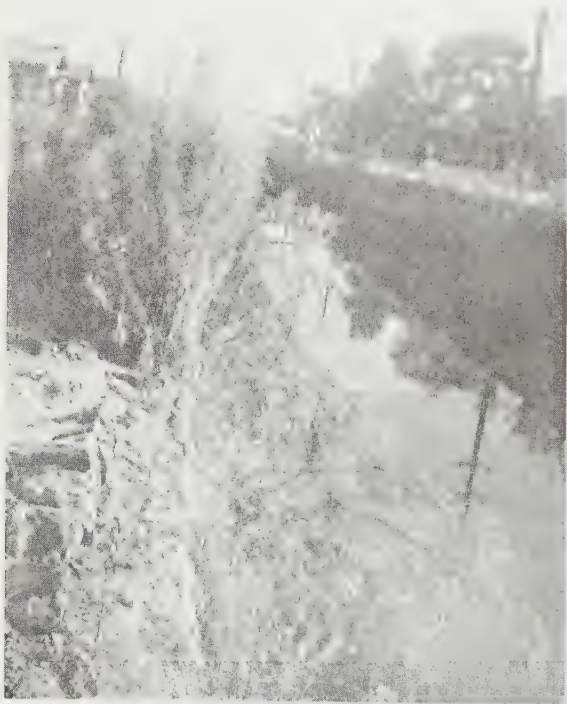


Fig. 2. Ditch wall vegetation constituted with *Aster subulatus* and three species of *Erigeron* (the 25th Sep. 1951).

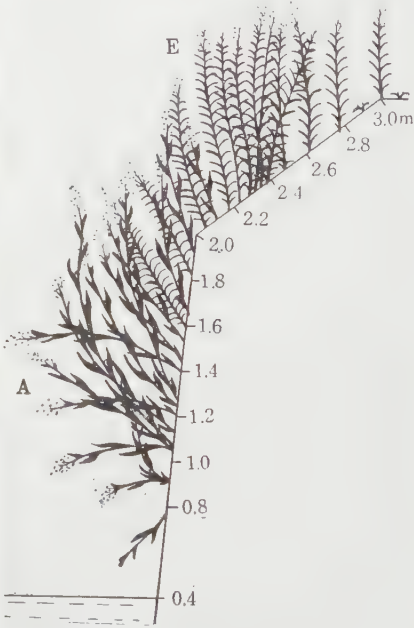


Fig. 3. Diagrammatic beisect through a ditch wall vegetation
A, *Aster subulatus*
E, *Erigeron* spp.

3) The pure communities at vacant ground

a. A comparison between accompanying species. In each of the pure communities of *A. subulatus* and of *E. sumatrensis*, 10 quadrats of 1 m² were set accord-

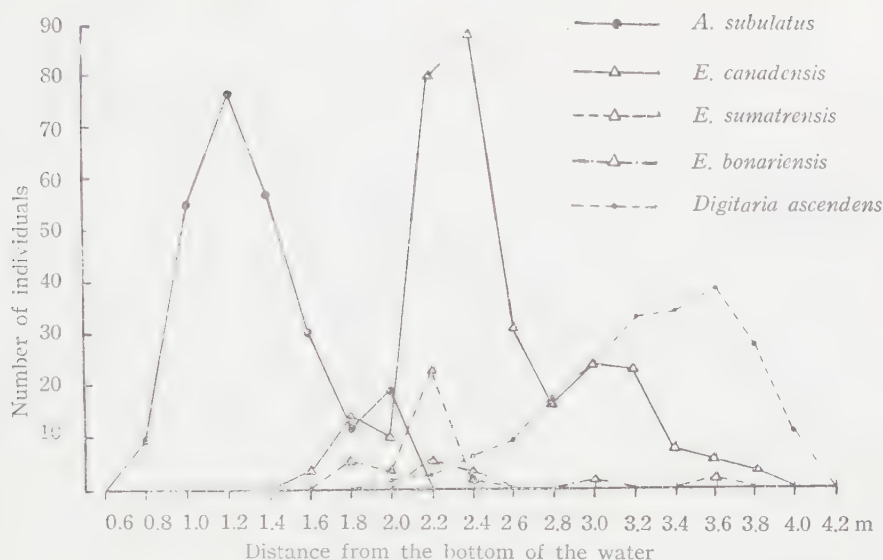


Fig. 4. Micro growing ranges of the main plants on a ditch wall

ing to the systematic sampling, and the accompanying species (which had been divided into 3 groups*: the wetter, middle and drier groups, according to their general character for a soil moisture condition) were compared between the two pure communities with respect to the percentage of species number, coverage and frequency. In the community of *A. subulatus*, the floristic percentage is 47% in the wetter, 29% in the middle and 24% in the drier group, while in the *E. sumatrensis* community, the wetter group is not found, and the drier one shows such a higher value as 80%. Furthermore, for their total coverage and frequency percentage the same tendency as in the case of the floristic percentage is observed. By the preliminary survey in September 1951 nearly the same results were obtained.

b. Comparison of the chemical components and the water content of the soil between the two communities. As it is clear in Table V, the results of chemical analyses of the soil show not very remarkable differences between the two pure communities in total nitrogen, humus, pH, P_2O_5 and CaO. On the other hand, although the soil belongs to the sandy roam type and humus were very few in both habitats, the maximum water holding capacity as well as the soil water contents both soon after and 3 days after a rain-fall shows higher values in *A. subulatus* community than *Erigeron* communities.

From the results mentioned above, it becomes clear that the *A. subulatus* and the *Erigeron* spp. communities exhibit a distinct heterogenous habitat segregation which is due to the soil moisture condition.

* These three groups are not those which are divided according to the so-called plant geographical view point^{6, 7)} but comparatively classified ones in view of the weed communities.

Table. III. A comaprison of accompanying species between two pure communities (I).

Species	<i>A. subulatus</i> community			Species	<i>E. sumatrensis</i> community		
	Character for moisture	C. d.	Freq.		Character for moisture	C. d.	Freq.
<i>Panicum Crusgalli</i> L. var. <i>submutica</i>	W	1.8	90	<i>Digitaria ascendens</i>	D	2.2	90
<i>Polygonum longisetum</i>	M	1.4	60	<i>Setaria viridis</i>	D	1.1	70
<i>Eclipta prostrata</i>	W	0.9	50	<i>Trifolium repens</i>	D	1.0	40
<i>Juncus tenuis</i>	M	0.8	40	<i>Oxalis corniculata</i>	D	0.9	70
<i>Rorippa islandica</i>	W	0.6	30	<i>Sagina japonica</i>	D	0.6	60
<i>Polygonum nodosum</i>	M	0.6	40	<i>Gnaphalium affine</i>	D	0.4	40
<i>Bidens tripartita</i>	W	0.5	30	<i>Artemisia asiatica</i>	D	0.4	10
<i>Polypogon fugax</i>	W	0.4	20	<i>Hydrocotyle martiana</i>	M	0.3	20
<i>Plantago asiatica</i>	M	0.4	20	<i>Euphorbia supina</i>	D	0.3	20
<i>Hydrocotyle maritima</i>	M	0.3	10	<i>Juncus tenuis</i>	M	0.3	20
<i>Digitaria ascendens</i>	D	0.2	20	<i>Chenopodium album</i>	D	0.2	20
<i>Chenopodium album</i>	D	0.2	20	<i>Fatoua villosa</i>	D	0.2	20
<i>Cyperus microiria</i>	W	0.2	20	<i>Stellaria neglecta</i>	M	0.2	20
<i>Alopecurus amurensis</i>	W	0.1	10	<i>Sonchus oleraceus</i>	M	0.2	20
<i>Rumex japonicus</i>	W	0.1	10	<i>Oxalis Martiana</i>	M	0.1	10
<i>Portulaca oleracea</i>	D	0.1	10	<i>Aster subulatus</i>		0.2	10
<i>Achyranthes japonica</i>	D	0.1	10	<i>Erigeron sumatrensis</i>		4.8	100
<i>Aster subulatus</i>		5.0	100	<i>E. canadensis</i>		2.1	80
<i>Erigeron sumatrensis</i>		0.6	50	<i>E. bonariensis</i>		0.5	30
<i>E. canadensis</i>		0.1	10				
<i>E. bonariensis</i>		0.1	20				

W, Wetter ; D, Drier and M, Middle group.

Table IV. A comparison of accompanied plants between two pure communities (II).

Groups by character for moisture	<i>A. subulatus</i> community (17 spp.)			<i>E. sumatrensis</i> community (15 spp.)		
	Numb. of spp. %	Total c. d. %	Total freq. %	Numb. of spp. %	Total c. d. %	Total freq. %
Wetter	47	53	55	—	—	—
Middle	29	40	32	20	9	12
Drier	24	7	13	80	91	88

The subject species not shown here: *A. subulatus*, *E. sumatrensis*, *E. canadensis* and *E. bonariensis*.

II. Homogenous habitat segregation of *A. subulatus* due to the soil moisture.

From the results of the field survey mentioned above, it is clear that there is a habitat segregation due to the soil moisture between the communities of *A. subulatus* and *Erigeron* spp. Namely, the main growing range of *A. subulatus* is always connected with higher moisture. There is often found a pure community of *A. subu-*

Table V. Chemical components and soil water content of the bed soil

Comm- unity	Sampling portion	Total nitrogen	Hu- mus	pH	P ₂ O ₅	CaO	Soil water contents		
							Soon after a rain-fall	3 days after a rain-fall	Maximum water capacity
<i>A. subulatus</i>	0—5 ^{cm}	0.29 [%]	1.26 [%]	5.5	200 ^{p.p.m.}	less than 0.07	19.7 [%]	17.9 [%]	38.4 [%]
	5—10	0.22	1.88	5.5	200	0.07—0.12	19.5	15.3	39.1
	10—15	0.22	1.66	6.5	200	less than 0.07	19.5	16.1	44.6
	15—20	0.14	1.32	7.0	150	less than 0.07	20.4	16.9	48.3
	20—30	0.10	1.12	7.0	150	less than 0.07	20.5	19.5	51.4
<i>E. sumatrensis</i>	0—5	0.29	1.22	6.0	200	0.07—0.12	15.7	12.0	37.7
	5—10	0.14	1.17	6.3	200	over 0.17	16.4	12.7	36.2
	10—15	0.07	1.12	7.0	100	over 0.17	17.5	12.5	37.6
	15—20	0.15	1.07	7.2	50	0.07—0.12	19.5	12.7	35.8
	20—30	0.11	1.01	7.5	20	0.07—0.12	18.9	13.8	39.5

Table VI. Anatomical comparison of subterranean part of *A. subulatus* from drier and wetter habitat (measuring individual number was 25 in each)

Root form (Habitat)	S (Drier)	Re or Br (Wetter)	Significancy
Root diameter mm	3.9±0.34	4.1±0.42	—
Cortex thickness μ	250±64	780±133	+
Ct. Q.	14±3.2	36±4.6	+
Diameter of wood parts mm	2.8±0.35	2.2±0.33	+
Radial number of vessel arrangement	24±3.6	20±4.4	+
Largest vessel diameter μ	106±29.3	74±17.9	+
Smallest vessel diameter	14±4.2	14±5.4	—
Vessel concentration (in 1 mm ²)	23±5.4	19±5.0	+
Intercellular space in cortex	none	presence	
Hardness of wood parts	hard	little soft	

Significancy of the differences: —none-significant, +significant at 1% level

latus where the *Erigeron* plants can hardly invade*. On the other hand, in the drier stand just adjoined to the wetter ground, a dense community of *Erigeron* spp. is found. This community is sometimes accompanied by a few individual plants of *A. subulatus*. With regard to the root form⁴⁾ of *A. subulatus*, it is observed that plants of *A. subulatus* mixed in the *Erigeron* community show a straight root form (S) in the same manner as in the case of *Erigeron* spp., but those of *A. subulatus* in its pure community show a reticulate form (Re) or branched root form (Br) (Fig. 5).

* According to the survey made on the 6th July, 1953, it was observed that the *A. subulatus* community encountered a flood owing to a long rain for 12 days and that some individuals of the accompanying *E. sumatrensis* and *E. canadensis* suffered several injuries, for example, the leaves were withered and the epidermis of roots were blacked and becoming off.



Fig. 5. Showing root form and anatomical features of *A. subulatus* from different habitats. **A** from wetter habitat (in its pure community) shows a reticulate root form, and **B** from drier habitat (accompanying individuals in *E. sumatrensis* community) shows a straight form. **A'** and **B'** are cross sections of the roots of **A** and **B** respectively at nearly the same parts in diameter. **A** and **B** $\times \frac{1}{4}$, **A'** and **B'** $\times \frac{1}{55}$

Furthermore, *A. subulatus* of the two communities exhibits clear differences in the anatomical features of root such as **Ct. Q***, the radial number of vessel arrangements, the diameter of the largest vessels, the diameter of wood parts, the vessel

* **Ct.-Q.** (=Cortex thickness-Quotient) represents the percentage of the cortex thickness for the root diameter of the same individuals; that is,

$$\text{Ct.-Q.} = \frac{\text{cortex thickness}}{\text{root radius}} \times 100$$

concentration, etc. Moreover, remarkable differences are found in regard to the presence of intercellular space in cortex and the hardness of wood parts.

On the relation between the root form and relative abundance in the plants in the treated area, the results as shown in Table VII were obtained.

Table VII. Root form and relative abundance in the plants in the treated area

Species \ Habitat	Wetter stand		Drier stand	
	Root form	Abundance	Root form	Abundance
<i>A. subulatus</i>	Re (Br)	abundant	S	seldom present
<i>E. sumatrensis</i>	—	—	S	very abundant
<i>E. canadensis</i>	—	—	S	abundant
<i>E. bonariensis</i>	—	—	S	present

Summary

1. The author experimentally ascertained a heterogenous habitat segregation due to the soil moisture between *Aster subulatus* and three species of *Erigeron*, *E. sumatrensis*, *E. canadensis* and *E. bonariensis*, both of which belong to the same life form and growing period.

2. The main growing range of *A. subulatus* is usually connected with higher soil moisture, but there is found a few individuals of *A. subulatus* accompanying in the community of *Erigeron* spp. which grows in drier habitat. The root form of *A. subulatus* in the *Erigeron* community shows a straight root form (S) as well as in the three species of *Erigeron*. On the other hand, the root form of *A. subulatus* in its pure community shows a reticulate form (Re) or branched root form (Br).

3. As to the anatomical features of root of *A. subulatus* from both communities, the pure communities of *Aster* and *Erigeron*, differences are clearly found in Ct-Q., presence of intercellular space, largest vessel diamter, etc., and the author recognized a homogenous habitat segregation between individuals of *A. subulatus*. Among the plants of *Erigeron* spp. were not found so notable differences as in *Aster subulatus*.

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Climatic and Growth Periodical Variation of Osmotic Value in Soybean Plants*

by Shosuke KAKU**

賀 来 章 輔: 大豆における気候及び生育週期的滲透値変化

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Fukuda and present writer (1952) reported that the osmotic value in soybean plants increased gradually as the plants grew and the maximum value appeared at the podding or at the ripening season. But the drop of osmotic value and also that of transpiration were observed at the beginning of flowering. But we could not determine whether the falling of osmotic value at flowering was affected by the external factor which caused the drop of evaporation as well as transpiration, or by the internal factor which directly affects the variation of osmotic value. The factor which affects the osmotic variation of plants is the seasonal succession of environmental factors^{4, 6)} and the growth periods^{2, 3, 6, 7)}. Fukuda's opinion¹⁾ is that season means for the plant the change of meteorological conditions and periods of growth. To analyse these two factors which affect osmotic variation, the present writer used soybean plants (*Glycine hispida*) as before. Two varieties, mid-season and late-season, were planted at the same time twice in the year. If in the same growth period of two varieties under different meteorological conditions, a similar periodical variation of osmotic value appears, the variation may depend on growth period. If, however, under similar meteorological conditions periodical variation of osmotic value appears similarly in two varieties the variation may depend on the change of meteorological conditions.

Material and Method

One variety of mid-season "Shakkinnashi" and two of late-season "Tamanishiki" and "Iyo," were used as materials. They were sown in pots as follows: "Shakkinnashi" and "Tamanishiki" on May 30, 1952 and "Shakkinnashi" and "Iyo" on July 20, 1952. Soil moisture was kept at either 80% (properly moist plot) or 20% (drought plot) of saturated water capacity. For the determination of osmotic value, the author measured the osmotic pressure of the epidermal cells of the 2nd~15th leaves of a plant at incipient plasmolysis in KNO₃ solution.

*Problem of physical and physiological dryness. Rep. 16 by Y. Fukuda

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Experimental Results

1) Time difference of flowing and podding between two varieties. A) Early summer sowing As denoted in table I, the plants of a moist plot began flowering on the 72nd day in the late-season variety and on the 60th day in the mid-season one and the difference between the two was 12 days. Podding began on the 66th day in the latter and on the 89th day in the former, the difference between the two being 23 days. On the other hand, in a drought plot the midseason variety matured earlier and the late one later than the one grown in a moist plot.

Table I. Growth period of soybean plants sown on May 30, 1952

Variety of soybean	Moist plot (80%)			Drought plot (20%)		
	Days before flowering	Days before podding	Difference	Days before flowering	Days before podding	Difference
Late-season	72	89	17	75	89	14
Mid-season	60	66	6	54	60	6
Difference of two varieties	12	23		21	29	

B) Mid summer sowing As denoted in table II, the plants sown in mid summer matured earlier than those sown in early summer but the relation between the mid-season and late-season is similar to that of those sown in early summer.

Table II. Growth period of soybean plants sown on July 20, 1952

Variety of soybean	Moist Plot (80%)			Drought plot (20%)		
	Days before flowering	Days before podding	Difference	Days before flowering	Days before podding	Difference
Late-season	45	59	14	53	63	10
Mid-season	31	37	6	36		
Difference of two varieties	14	22		17		

2) Seasonal changes of osmotic value according to growth periodicity. The osmotic value of the plants of properly moist plot was indicated by thick lines in figs. 1 and 2. The tendency of osmotic variation does not differ according to the difference of variety and the time of sowing. It gradually increases as the plants grow and attain to the maximum value at ripening. But once before flowering there appears a sudden descent of osmotic value which again ascends at podding. This periodical variation similarly occurs in two varieties of mid and late-season ones although both mature under different meteorological conditions.

3) Seasonal changes of osmotic value according to the meteorological conditions. The osmotic value on the drought plot was indicated by thin lines in figs. 1 and 2. A growth periodical osmotic variation is observable both in the plants of properly moist plot and of drought plot before podding, but in the late period of growth, except that of the withered one sown in mid summer, it gradually decreases

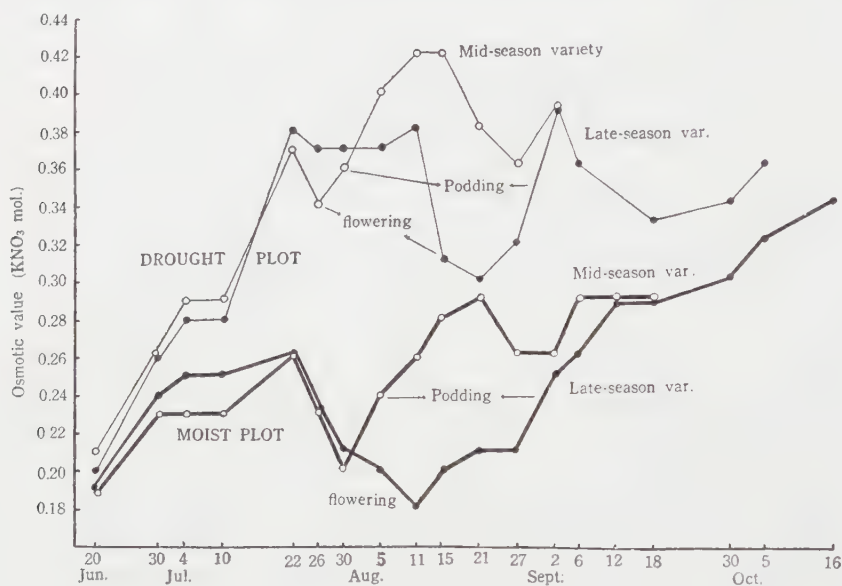


Fig. 1. Osmotic value of soybean plants sown in early summer

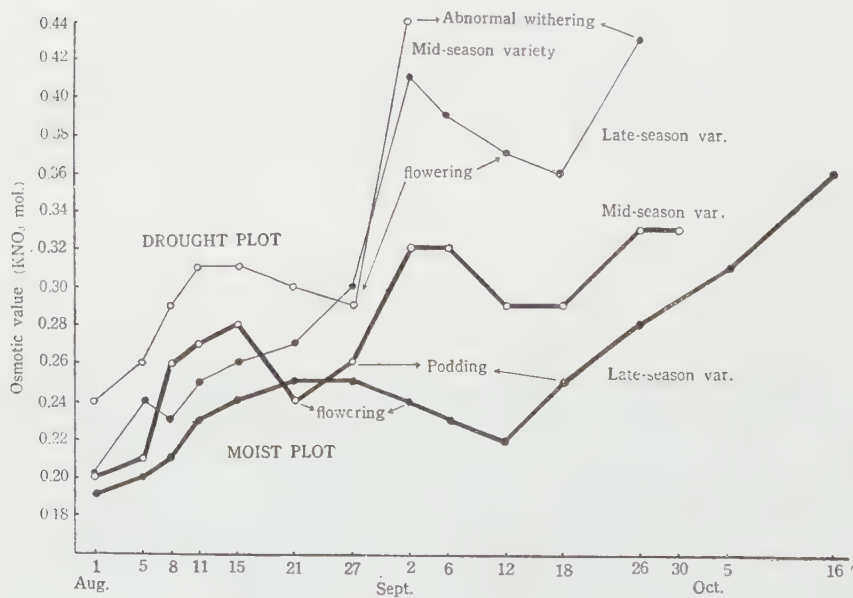


Fig. 2. Osmotic value of soybean plants sown in mid summer

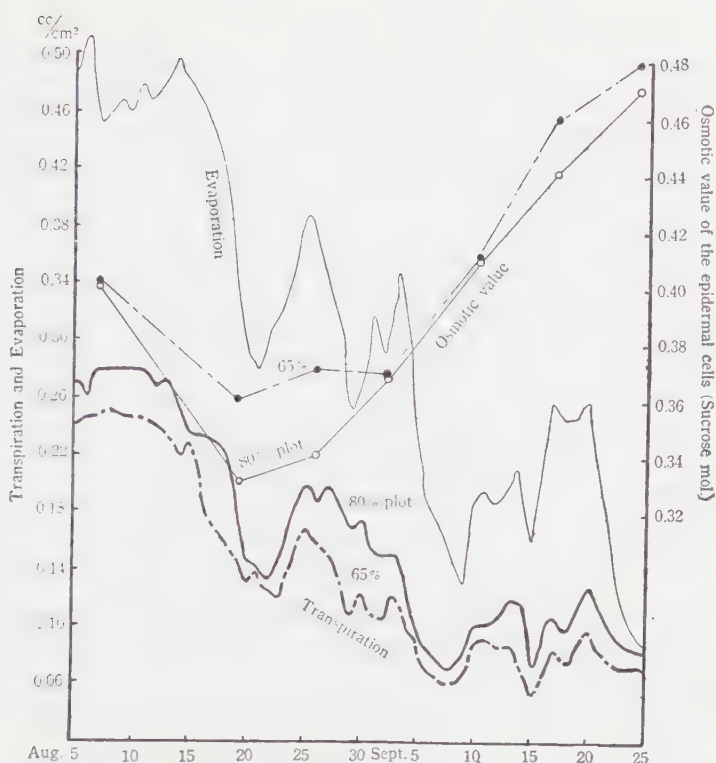


Fig. 3. Variation of transpiration, evaporation and osmotic value of soybean plants (recomputed from original data of 1952)

and approaches the value of moist plot. This agrees with the result of the former report (1952). This gradual decrease in the late period of growth in contrast with the former forced ascension of osmotic pressure on a drought plot seems due to the osmotic regulation in accordance with the meteorological conditions which becomes cooler in autumn.

Discussion

When we take the shifting average of six day's periods of the data in 1952, it can be confirmed that the decrease of transpiration accompanied the reduction of evaporation as indicated in fig. 3. So that the decrease of transpiration was not caused by the coming of flowering season but by the change of meteorological condition which also caused the decrease of evaporation. The descent of osmotic value which was observed at that time was not the phenomenon corresponding to the decrease of transpiration, but one which might happen at the flowering season. Spaning⁸⁾ and Suzuki⁹⁾ reported that the plant assimilates rhythmically in harmony with growth process. Kôriba⁵⁾ stated that assimilation diminishes in the midst of flowering. The present experiments proved that two varieties, mid-season and late-season, which have different growth periodicity show similar growth periodical osmotic

variation under different meteorological conditions. On drought plots, however, the osmotic value excessively ascends during the summer when the transpiration is very strong but that excessive ascension releases after podding and gradually it descends. The similar phenomenon was also observed formerly²⁾. It may be the seasonal osmotic regulation caused by the meteorological changes. In two varieties sown late on the drought plot, the osmotic decrease at the flowering season passed off well, but the following ascent of osmotic value caused the abnormal withering of the plant. The withering of the mid-season variety occurred in summer when the meteorological condition required high osmotic value. But the withering of the late-season variety occurred in autumn, although the meteorological condition at that time did not require high osmotic value even in drought soil. Therefore it seems that the ascension of osmotic value which depends on the growth period surpasses the descension which ought to occur in accordance with the cooler meteorological condition.

Conclusion

Three varieties of soybeans which have different growth periodicity were cultured twice in different meteorological seasons, and the author succeeded in analysing the seasonal osmotic variation of soybean plants into two periodical factors, the growth periodicity and climatic one. According to the growth periodicity, osmotic value gradually increases as the plant grows, but during the growth period it descends once at flowering and ascends again at podding although such variation delays when the process of growth retards. The excessive ascension of osmotic value on drought plots in summer when transpiration is strong and the release of ascension of it in autumn when transpiration becomes weak may be due to the climatic periodicity. And this climatic factor can only effect secondarily to the seasonal osmotic variation and the growth periodicity dominate it.

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Cytological and Morphological Studies on the
Gametophytes of Ferns IX
The Polar Plasmolysis on Fern-prothallium (1)*

by Isami IGURA**

伊倉伊三美：羊齒類の配偶体に関する細胞学的並に形態学的研究 IX
羊齒類前葉体の有極性原形質分離 (1)

Received February 2, 1955

The cyto-physiological studies on the fern-prothallia have been carried out by Gratzy-Wardengg⁸⁾ and by Reuter¹⁸⁾. These authors determined the osmotic values (limit concentrations of the plasmolytica) of saccharose or glucose in some species of fern-prothallia which were cultured under certain external conditions and confirmed the existence of gradients of the osmotic values in the case of prothallium. Orth¹⁷⁾ made the morphological and physiological researches on the fern-prothallia but not on the plasmolyses of these prothallial cells. Besides the investigations on the prothallia, Esterák³⁾ researched that the leaves of *Elodea canadensis* showed the "Grundgradienten" which is caused by the physical and chemical conditions and reported also in reference to the prothallium-gradient. In the *Helodea*-leaves fixed with 70% alcohol, Drawert¹⁾ recognized that they revealed the existence of gradients in stainability and the correlation between the isoelectric point of the different portions of the cells and the degrees of differentiations of them. Yamaha and Negoro^{13~25)} studied the osmotic phenomena on *Oscillatoria princeps* and reported the action of several plasmolytica on the plasmolysis. Recently, the differential plasmolysis in the eggs of *Coccophora* and *Sargassum* or the plasmolytic polarity in *Cladophora* was investigated by Nakazawa^{15, 16)}.

In this study the author intended to determine the existence of different osmotic value and the isotonic or the permeability coefficient also according to the portion of the adult prothallium which was not cleared by any other investigators of the fern-prothallia. With respect to the phenomenon of plasmolysis such as its form, duration, and deplasmolysis which appeared in the cells of the whole prothallium or in a single prothallial cell, new data were found, so they will be described in the following.

* a) The summary of this study was reported at the 19th annual meeting of the Botanical Society of Japan held on the 26th to 28th October, 1954 at Kyoto University. 2) The expences for the present study were partly paid by the Grant in Aid for the Micellaneous Scientific Research from the Ministry of Education.

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Materials and Methods

The prothallia of Pteridophyta used in the present study, were the following species belonged to Polypodiaceae: *Asplenium incisum* Thunberg, *Leptogramma totta* J. Smith, *Leptorumohra Miqueliana* H. Ito, and *Thelypteris japonica* Ching. The spores were sown on the sphagnum and a piece of unglazed pot in the Petri-dishes which were sterilized on December, 1952. These Petri-dishes were kept in the thermostat at 22°C and were able to receive the light normally in the room. The spores germinated in from eight days to two weeks culture and developed into the prothallia. In most cases the adult prothallia were used. As the plasmolytica, the following reagents were employed: nonelectrolytes (ethyl alcohol, glucose, glycerin, saccharose, and urea) and electorolytes (AlCl_3 , CaCl_2 , KCl , KNO_3 , MgCl_2 , NaCl , Na_2SO_4 , NH_4Cl , HCl , and $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$). These reagents were applied as mol.-solutions at intervals of 0.1 or 0.02 M dissolved in the redistilled water and the solutions were reserved in the hard glass bottles. The slide- and the cover-glasses were immersed in the solution of the chrom-sulphuric acid for about twenty-four hours and washed in the running water for about the same time. The most preparations were made by the following method. The paraffin was set at the both sides of a drop of the plasmolyticum in which the prothallia were dipped and the cover-glass was put on the drop and the paraffin, and then the border of the cover-glass was sealed with

the paraffin. By means of this method the concentration of the plasmolyticum on the slide-glass was kept constantly for a long time during the observation. The prothallium was taken care of to prevent its injury when it was treated. Thus, the preparations were investigated under the ordinary microscope or the phase contrast one.

Experimental Results

The prothallia of four species used passed the filamentous protonema stages and developed into the heart-shaped forms respectively, each of which consists of the symmetrical wings, the apical notch (meristem), the protonema, and the rhizoid, and the prothallia of *Leptorumohra Miqueliana* H. Ito and *Thelypteris japonica* Ching possess the glandular hairs (papillae) on the border and the sur-

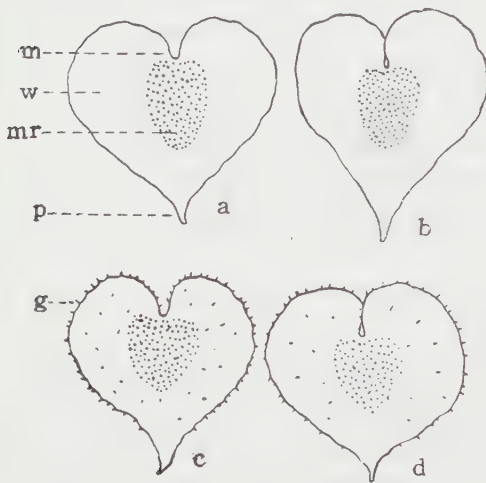


Fig. 1. The outlines of the morphological features of the adult prothallia employed in the experiment. (\times ca. 10). The prothallial cell, the archegonium, the antheridium, and the rhizoid are not shown. m, meristem; mr, midrib; w, wing; g, glandular hair; p, protonema. a, *Asplenium incisum* Thunberg; b, *Leptogramma totta* J. Smith; c, *Leptorumohra Miqueliana* H. Ito; d, *Thelypteris japonica* Ching.

face of the prothallial cells here and there (Figs. 1 a, b, c, d)^{9, 11, 12, 13, 14, 19).}

In this study it is considered to be pertinent to distinguish the surface area of the prothallium into several regions according to the stage of development and growth of the prothallial cells and to classify it into six regions, viz. Region I, II, III, IV, V, and VI (Fig. 2 a), though Gratzy-Wardengg⁹⁾ and Reuter¹⁸⁾ had disting-

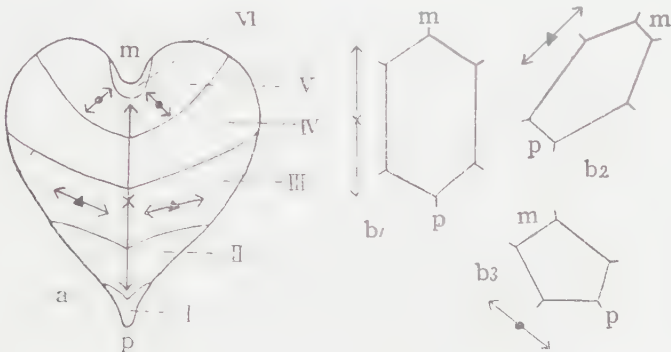


Fig. 2. The schematic views which indicate six regions and the orientations of three polarities marked with allows. a, whole surface area in the prothallium; b, single prothallial cell; m, apical pole; p, basal pole; \leftrightarrow , longitudinal polarity; $\leftarrow\bullet\rightarrow$, radial polarity; $\leftarrow\blacktriangle\rightarrow$, tangential polarity. The lines which divided the regions were drawn generally according to the orientation of the row of the prothallial cell.

uished five zones. Moreover, the author considered the existence of the polarities in the whole prothallium or within a single cell, too. This polarity seems to be caused by the difference of the stage of development and growth of the prothallial cell and is found in the following three directions: 1) Longitudinal (“medial” of Reuter), 2) Tangential, 3) Radial. Judging from the present experiments, the author corroborated that this fact was reasonable as Reuter¹⁸⁾ stated. At the longitudinal polarity the author tentatively the name of a basal pole (protonema-pole) and an

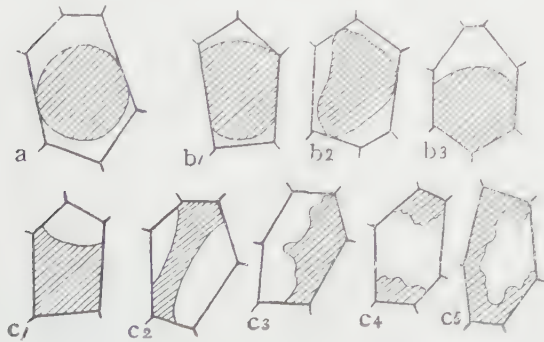


Fig. 3. The forms of plasmolysis of a single prothallial cell ($\times 250$). To the portion of the cytoplasm the oblique lines were added. a, A-type; b₁–b₃, B-type; c₁–c₅, C-type.

apical pole (meristem-pole) to the portion of the protonema and the meristem (growing point in the apical) respectively (Fig. 2 a), and the cells of the former are old, whereas those of the latter are young. In a single cell also two poles above-mentioned were considered by the author provisionally (Figs. 2 b₁, b₂, b₃).

The forms of plasmolyses of the prothallial cells are classified into three types. The first case

is the one which the cytoplasm shrinks in a round shape (that is, convex type), in other words, the plasmolysis gets to the maximum state and this is called A-type by the writer, secondarily, the cytoplasm separates from the membrane merely in the convex state and this is B-type, and thirdly the shrinkage of the cytoplasm is concave or irregular state and this is C-type (Fig. 3).

The experimental results will be reported in the following five contents: 1) The from of plasmolysis, 2) The duration of plasmolysis (plasmolysis time), 3) The osmotic value, the isotonic and the permeability coefficient, 4) The permeability—The deplasmolysis, 5) Behaviour of the plastid.

The form of plasmolysis

The form of plasmolysis of the prothallial cell is different according to the region and this difference is distinct at the longitudinal polarity within the whole field of the prothallium. The extracts of the results are given in the following Table I.

Table 1. The form of plasmolysis at each region in the whole field of the prothallium

Plasmo-lytica	Species	Mol.	Times (mins.) (after)	Regions						Remarks
				I	II	III	IV	V	VI	
	Ai	0.30	20	±B	±B	±±B	—	—	—	I-27; 14°, 10°C
		0.38	15	+A	+A	±AB	±±B	—	—	
		0.82	90	+A	+A	+BC	+BC	+BC	+CB	
	Tj	0.32	8	±±±B	±±±B	—	—	—	—	II-6; 12°, 7°C
		"	30	±B	±±B	—	—	—	—	
		0.40	30	±B	±B	±±B	±±±B	—	—	
		0.86	20	+C	+C	+CB	+B	±B	±±B	
	Saccharose	Lt	0.22	20	—	—	—	—	—	II-23; 18°, 5, 12°C
			0.34	5*	±±±B	±±±B	—	—	—	
			"	10	±B	±B	±±BC	—	—	
			0.58	20	+BC	+BC	+BC	±BC	±±BC	
		Lm	0.38	5*	±±±B	—	—	—	—	II-16; 19°, 12°C
			"	7	±±B	±±B	—	—	—	
			"	11	±B	±B	±±B	—	—	
			"	15	±B	±B	±±B	±±±B	—	
			"	30	+BA	+BA	±BA	±±BA	—	
			1.00	20	+BC	+BC	+BC	±BC	±BC	±±BC

Plasmolytica	Species	Mol.	Time (mins.) (after)	Regions						Remarks
				I	II	III	IV	V	VI	
	Ai	0.30	3	+B	±B	±B	±±B	—	—	II-5; 17°, 8°.5C
		"	10	+B	+B	+B	±B	—	—	
		"	25	+BA	+BA	+B	±B	±±B	—	
	Tj	0.16	20	±B	±B	±±B	—	—	—	II-13; 19°, 10°C
		0.32	2*	+B	+B	—	—	—	—	II-15; 15°, 9°C
		"	10	+B	+B	±B	±±B	—	—	"
		"	20	+B	+B	±B	±±B	±±±B	—	"
		"	30	+B	+B	+B	±B	±±B	—	"
	CaCl ₂	0.12	10	±±B	±±B	—	—	—	—	III-6; 18°.5, 10°C
		0.30	4*	±±±B	±±±B	—	—	—	—	
		"	10	+BC	+BC	±BC	±±B	—	—	
		"	30	+BC	+BC	+BC	+BC	+BC	—	
		0.40	20	+B	+B	+B	±B	±±B	—	II-20; 19°.5, 12°C
		0.20	10	±±B	±±B	—	—	—	—	
		"	20	±B	±B	±±B	±±B	—	—	
		0.40	4*	±±B	±±B	±±±B	—	—	—	
	Ai	"	15	+A	+A	+AB	±AB	±±B	—	III-2; 18°, 14°C II-27; 15°, 11°C " "
		0.42	15	±±B	±±B	—	—	—	—	
		1.00	4	+B	+B	—	—	—	—	
		"	7	+B	+B	±B	±±B	—	—	
	Tj	"	10	+A	+A	+AB	±AB	±BA	±±±BC	III-6; 18°5, 10°C
		0.30	15	—	—	—	—	—	—	
		0.40	16	±B	±B	±±BC	±±±BC	—	—	
	Li	0.70	20	+A	+A	+AB	±AB	±BA	±±±BC	III 6; 18°.5, 10°C
		0.38	15	±BC	±BC	±±BC	±±±BC	—	—	
		0.60	1*	±±±BC	±±±BC	—	—	—	—	
		"	5	±B	±B	±BC	±±BC	±±BC	—	
		"	15	±BA	±B	±BC	±±BC	±±BC	—	
		"	30	+A	+A	+AB	±BC	±±BC	—	
	Ai	0.90	15	+BA	+BA	+B	+B	±B	±±B	III-12; 9°.5, 12°C
		0.30	10	+B	+B	±B	±B	±±B	—	
		"	25	+B	+B	+B	+B	±BC	—	
	Tj	0.40	0.5	±B	±B	±B	±±B	—	—	III-15; 16°, 11°.5C
		"	7	+BA	+BA	+BA	±B	±±B	±±±B	
		1.00	5	+B	+B	±B	±B	±±B	±±B	
	Tj	0.18	9*	±±B	±±±B	—	—	—	—	III-15; 16°, 11°.5C
		0.26	10	±B	±B	±B	±±B	—	—	
		0.36	17	+A	+A	+B	±B	±±BC	—	

Plasmolytica	Species	Mol.	Times (mins.) (after)	Regions						Remarks
				I	II	III	IV	V	VI	
KNO ₃	Ai	0.16	7*	± ± ± B	± ± ± B	—	—	—	—	III-16; 16°, 11°.5C
		"	17	± ± B	± ± B	± ± ± B	—	—	—	
		0.60	6	+BA	+BA	+B	± B	± ± B	± ± ± B	
	Tj	0.22	15	± B	± B	± ± B	—	—	—	III-17; 17°.5, 11°C
		0.36	8	+BA	+BA	± B	± ± B	± ± ± B	—	
		"	30	+A	+A	+A	+A	+A	+A	
Glucos ³	Ai	0.46	2*	± ± ± B	± ± ± B	± ± ± B	—	—	—	III-20; 17°, 13°C
		"	20	± B	± B	± B	± ± B	—	—	
		0.68	15	+B	+B	± BC	± ± BC	± ± ± B	—	
	Tj	0.40	13	± B	± ± B	± ± ± B	—	—	—	III-22; 22°, 14°C
		0.68	15	+BC	+BC	± BC	± C	± ± C	± ± ± CB	
Gly-cerin		0.90	7	± B	± B	± B	± ± B	± ± B	± ± B	IV-20; 14°, 13°C
		0.30	60	—	—	—	—	—	—	
AlCl ₃		0.30	7	± ± ± B	± ± ± B	—	—	—	—	IV-30; 17°.5, 16°C
		0.34	30	± B	± B	± ± B	—	—	—	
		1.00	5	± ± BC	± ± BC	± ± BC	± ± BC	± ± BC	± ± ± BC	
MgCl ₂		0.32	12	± ± ± B	± ± ± B	—	—	—	—	V-7; 17°, 16°C
		0.52	5	± B	± B	± B	± ± B	± ± ± B	—	
		1.00	8	+A	+A	+A	+AB	+B	± BC	
Na ₂ SO ₄	Ai	0.60	10	± B	± B	± ± B	± ± ± B	—	—	V-11; 15°, 14°C
		"	20	± A	± A	± AB	± ± B	± ± ± B	—	
		0.90	10	+A	+A	± BA	± ± B	± ± B	—	
NH ₄ Cl		0.30	6	± ± B	± ± B	± ± ± B	—	—	—	V-15; 16°, 15°.5C
		1.00	5	+BA	+BA	± B	± B	± BC	± BC	
HCl, Ethyl alcohol		0.05 or 0.1-1.0		—	—	—	—	—	—	V-17; 18°, 17°C V-18; 19°, 18°C

Foot-note: 1) Ai *Asplenium incisum* Thunberg, Tj *Thelypteris japonica* Ching, Lt *Leptogramma totta* J. Smith, Lm *Leptorumohra Miqueliana* H. Ito. 2) In the column of Remarks, the date, the room- and water-temperature were represented respectively, e. g. I-27 means the 27th, January. 3) Marks +, ±, and — show the degrees of the plasmolysis, and + perfect or almost perfect, ± slight, ± ±, ± ± ± very slight, and — negative. 4) * The time when the incipient plasmolysis occurs.

(to be continued)

Electron-microscopical Study on Fine Structures of Diatom Frustules XIII

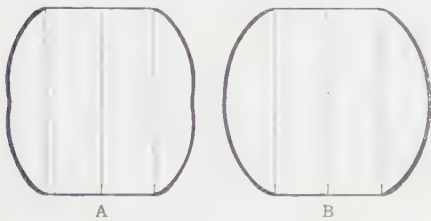
by Haruo OKUNO*

奥野春雄：電子顕微鏡による珪藻殻微細構造の研究 XIII

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Coscinodiscus concinnus W. Smith (Text figs. 1, 2-A, 3; Pl. I, figs. 1-6), Synop. Brit. Diat. **2**, p. 85 (1856); A. Schmidt, Atlas, pl. 114, figs. 8, 9 (1886); Hustedt, Kieselalg. **1**, p. 441, figs. 241-242 (1930); Mills, Index Diat. p. 463 (1933); Cupp, Bull. Scripps Inst. Ocean. **5**, p. 58, fig. 22 (1943); Cleve-Euler, K. V. A. Handl. **2**, no. 1, p. 68, figs. 108 a-f (1951).

L. M. S.¹⁾ Frustules drum-shaped, 200-380 (150-500) μ in diameter, with convex, in the center sometimes slightly depressed, valve surface (Text fig. 1). Pervalvar axis about 200-330 μ long, girdle about 50-90 μ high. Valves in dry preparation bright brownish yellow. In our specimens, central rosette distinct (Pl. I, fig. 1). Frustule pores arranged in radiating and secondary spiral rows, 9-10 in 10 μ , decreasing to 12 at the margin. Radiating hyaline lines and marginal spinulae distinct, about 1 in 10 μ . Two small asymmetrical processes present.



Text fig. 1. Two types of frustules of *Coscinodiscus concinnus*. A, Frustule with depressed valve-center. B, Frustule with non-depressed valve-center.

Preparation: Direct preparation without

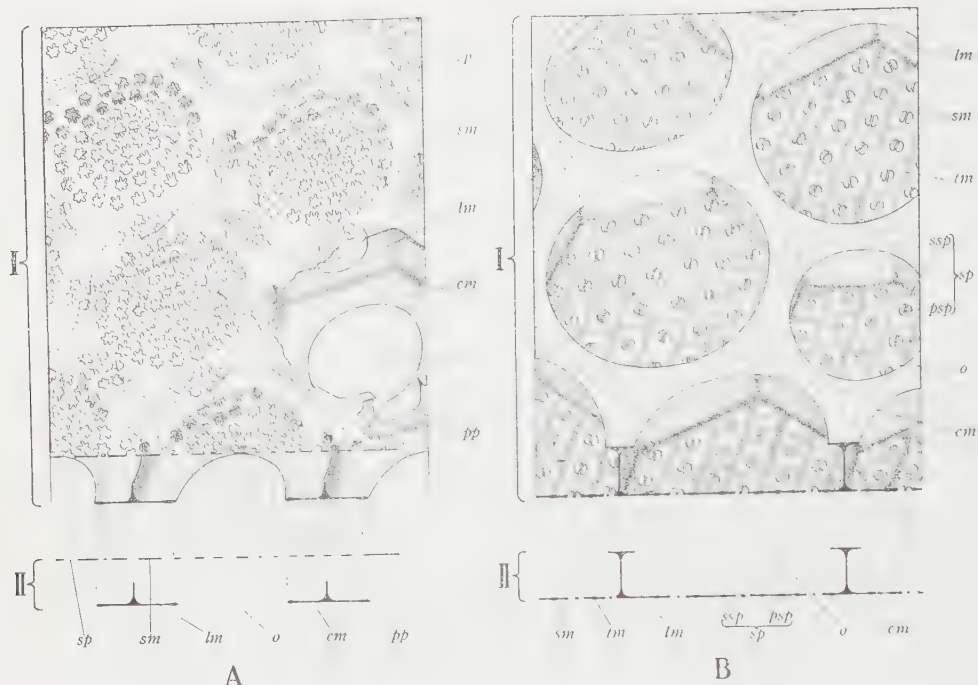
thermal or chemical treatment.

E. M. S.²⁾ Frustule pores in the valve surface are loculi. The loculus regular or scalene hexagonal (one side about 0.4-0.6 μ long), with outer sieve, inner closing, and lateral membranes (Text figs. 2-A, 3). The sieve membrane (*sm*), except on its margin has many sieve pores (*sp*). Sieve pores about 10-13 in 1 μ , triangular to polygonal with irregularly serrate borders, and arranged in somewhat concentrical rows. The inner closing membrane (*cm*) is very thin (somewhat penetrable to the electron beam) and has a central round opening (*o*) about 0.4-0.55 μ in diameter. The inner border of the closing membrane is electron-optically a little thicker than the other part. The lateral membrane (*lm*) is about 0.2-0.5 μ high, six-sided, each

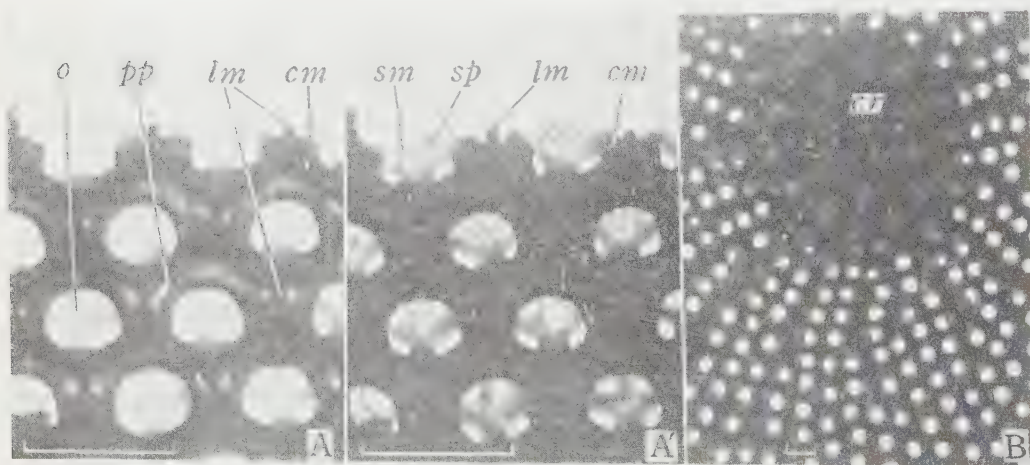
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1) L. M. S.: Light-microscopic structure

2) E. M. S.: Electron-microscopic structure



Text fig. 2. Diagrammatic representation of loculi of *Coscinodiscus concinnus* (A), and *Stephanopyxis palmeriana* (B). I, View from obliquely above. II, Longitudinal (perivalvar) section. *cm*, Closing membrane. *lm*, Lateral membrane. *o*, Opening of closing membrane. *pp*, Pass pore. *psp*, Partition of secondary sieve pore. *sm*, Sieve membrane. *sp*, Sieve pore. *ssp*, Secondary sieve pore. *tm*, Thickening of sieve membrane.



Text fig. 3. *Coscinodiscus concinnus*. A, A', Same portion of a broken valve (A, Light print. A', Dark print). B, Central part of valve. *cm*, Closing membrane (inner membrane). *cr*, Central rosette. *lm*, Lateral membrane. *o*, Opening of closing membrane. *pp*, Pass pore. *sm*, sieve membrane (outer membrane). *sp*, Sieve pore. (Electron micrographs. Scales: 1 μ .)

side with an angular pass pore (*pp*) about $0.1\text{--}0.2\mu$ broad, through which the neighbouring loculi communicate with each other.

Loculi in the girdle are about 18–20 and 20–22 in 10μ respectively in longitudinal and oblique rows. Loculi hexagonal, with outer and inner round opening respectively about $0.2\text{--}0.25\mu$ and $0.15\text{--}0.2\mu$ in diameter. The outer opening of the loculus lacks the closing sieve membrane.

Habitat: Marine, planktonic. Osaka Bay (Off the coast of Kariya, Awaji Island). (Okuno, No. m863. Aug. 1953).

Stephanopyxis palmeriana (Greville) Grunow (Text fig. 2-B; Pl. II, figs. 1a-e), Okuno, Bot. Mag. Tokyo, **63**, p. 97, pl. 1, fig. 1' (1950); Cupp, Bull. Scrips Inst. Ocean. **5**, p. 40, fig. 4 (1943).

Preparation: Direct and formval preparations without thermal or chemical treatment.

In my paper (1950) listed above, I reported some electron-microscopic structure of the porous sieve membrane of the present species. By my recent research, further details of fine structure of the loculus were revealed under the electron microscope. Here the new details in addition to the general structure will be presented. Loculi are large in the valve, small in the mantle, and smallest near the girdle line, respectively about 1.5–3, 4–5, 5–6 in 10μ . Loculi usually hexagonal, at the girdle line exceptionally pentagonal, *all opening outwards*³⁾, almost freely, and closing inwards by finely porous sieve membranes (Text fig. 2-B; Pl. II, fig. 1d). The outer closing membrane (*cm*) of the loculus marginal and narrow; in the center of the valve surface it is remarkably reduced in breadth and indistinct (Pl. II, fig. 1b). The central opening (*o*) of the outer closing membrane usually round to elliptic; in the valve surface it is large and nearly polygonal. The lateral membrane (*lm*) of the loculus very thin, not porous, and about $0.4\text{--}0.5\mu$ high. The inner sieve membrane (*sm*) has netveined rectangular, rarely polygonal thickenings (*tm*). The thickenings or the meshes in the whole mantle are arranged in common longitudinal parallel rows about 4–5 in 10μ , and in the whole valve surface they are arranged in common radiating rows about 4–5 in 10μ . Both in valve and mantle, the arrangement of thickenings or meshes is quite independent of that of the loculi. A mesh, in its center, has a round to elliptic sieve pore (*sp*) about $50\text{--}100m\mu$ in diameter, which is divided by a delicate partition (*psp*) into two semicircular secondary sieve pores (*ssp*). In the mantle, many of the partitions are parallel to the pervalvar axis, and in the valve, usually parallel to the radius of the valve. At the girdle line (*gl*), each of the sieve membranes of about every second to fourth loculi has a linear stigma about $700m\mu$ long and about $100m\mu$ broad (Pl. II, fig. 1e-st). Spines at the valve margin are hollow, and their walls are electron-optically non-porous (Pl. II, fig. 1c).

Habitat: Marine, planktonic. 39°N ; 153°E . (Okuno, No. m 977–8. Nov. 1952. Collected by R. Marumo.)

3) Cf. Cleve-Euler. K. V. A. H. **2**, no. 1, p. 36 (1951)

Stephanopyxis nipponica Gran and Yendo⁴⁾ (Pl. II, figs. 2a, b)

This species differs from *St. palmeriana* mainly in its smaller, oblong or sub-spherical frustules, but electron-optically is almost the same in the structure of loculi as *St. palmeriana*. In the present electron-microscopy, the netveined thickenings of sieve membranes and the partitions in sieve pores were not found. Stigma of the loculus at the girdle line is papillar, quite different in shape from that of the previous species. Spines at the valve margin are hollow, and their walls are non-porous as in the previous species.

Habitat: Marine, planktonic. 50°54'N; 178°53' W. (Okuno, No. m698. May, 1952). 41°58'N; 145°40'E. (Okuno, No. m975-6. Jan, 1954). (Collected by R. Marumo).

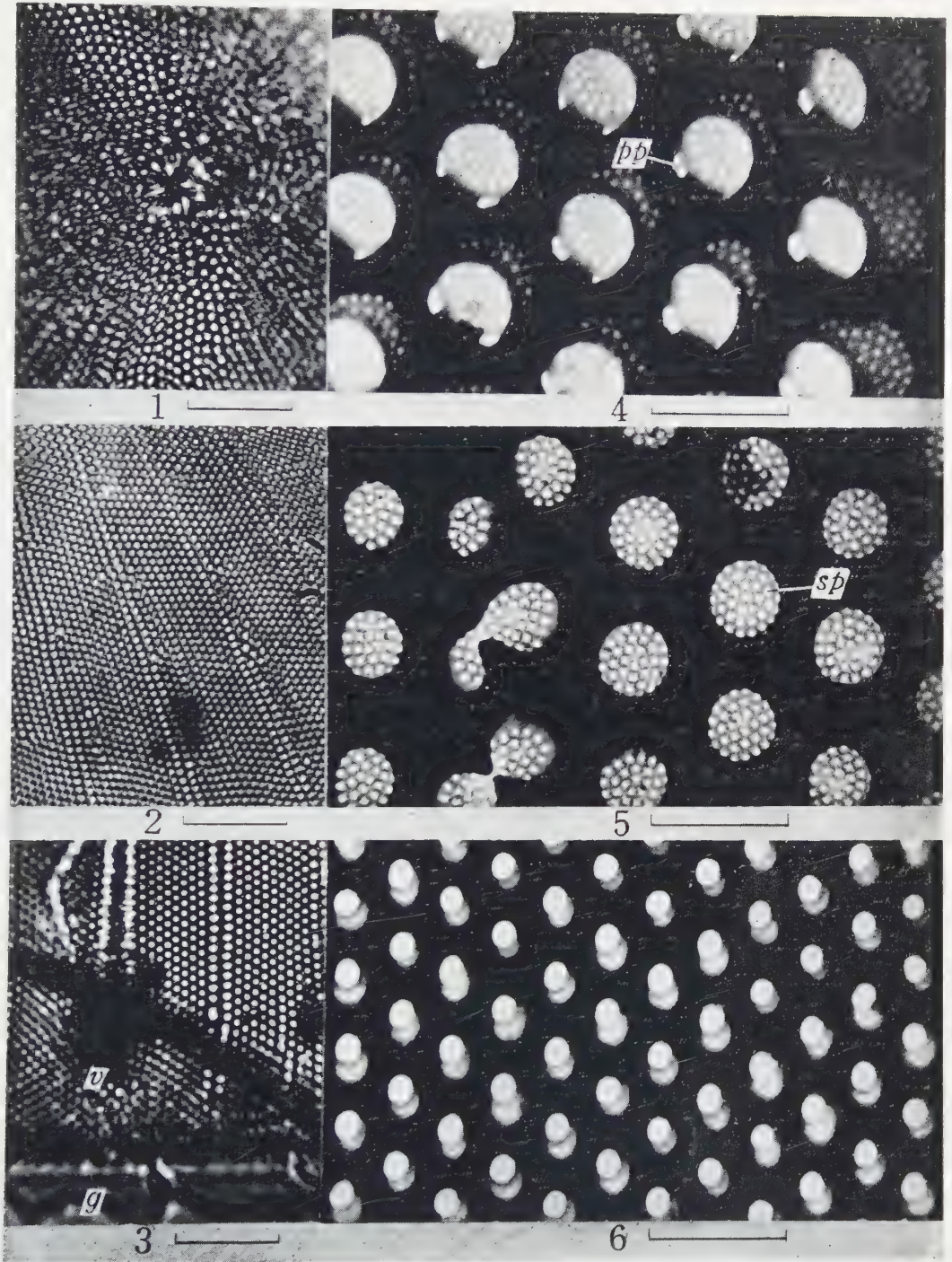
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5) Cf. This Magazine, 62: 139 (1949), 63: 106 (1950), 67: 177 (1954)

6) The present writer has not yet seen these publications.



Figs. 1-6, *Coscinodiscus concinnus* 1, Central part of valve, showing central rosette. 2, Part of valve near central area, showing 'hyaline lines'. 3, Portion of margin of valve (*v*) and girdle (*g*). 4, Portion of valve, viewed obliquely from inside; note the pass pore (*pp*) of loculus. 5, Portion of valve, viewed vertically; note the sieve pore (*sp*). 6, Portion of girdle viewed obliquely from inside, showing loculi. (1-3, Light micrographs. Scales: 10 μ. 4-6, Electron micrographs. Scales: 1 μ.)

H. Okuno: Fine structure of diatom frustules



Figs. 1a-e, *Stephanopyxis palmeriana*. 1a, Girdle view. 1b, Central part of valve. 1c, Spines. 1d, Portion of mantle. 1e, Loculus with stigma (*st*) near the girdle line (*gl*). 2a, b, *St. nipponica*. 2a, Girdle view. 2b, Portion of valve; note left side where broken loculi opening outwards are demonstrated. *cm*, Closing membrane. *gl*, Girdle line. *lm*, Lateral membrane. *sm*, Sieve membrane. (1a, 2a, Light micrographs. Scales: 10 μ . 1b-e, 2b, Electron micrographs. Scales: 1 μ .)

Studien über Anthocyane, XXVI¹⁾ Über den Farbstoff der Blüten von *Lespedeza Thunbergii**

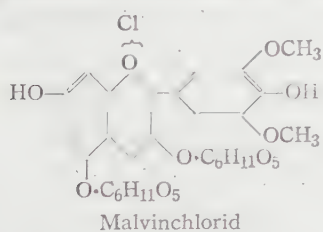
Von Kôzô HAYASHI, Tatsuo NOGUCHI und Yukihide ABE**

林孝三・野口辰男・阿部幸頼： アントシアン色素の研究(第26報), ミヤギノハギの花の色素

Eingegangen am 4. März 1955

Die strauchartigen Gewächse von *Lespedeza*, einer Gattung von Leguminosen, stellen wegen ihres graziösen Wuchses und der blütenreichen, nickenden, weissen oder rosa- bis purpurrotfarbigen Rispen eine bewunderte Zierde der herbstlichen Landschaft in Japan dar. Unter diesen hat *Lespedeza Thunbergii* NAKAI violettrote Blüten.

Um der chemischen Natur des Blütenfarbstoffs dieser Pflanze näher zu treten, haben wir uns mit Versuchen zu dessen Darstellung und Konstitutionsermittlung beschäftigt. Bei der Aufarbeitung der frischen Blüten sind wir auf nicht mindere Schwierigkeiten gestossen, da der Farbstoff von überschüssigem Bleiacetat leicht angegriffen wurde. Nach mehreren vergeblichen Versuchen ist es aber uns erst geglückt, den Farbstoff in prächtig krystallisiertem Zustand zu isolieren. An Hand dieses Präparates wurden chemische Analysen erfolgreich durchgeführt. Die Ergebnisse sprachen dafür, dass der Farbstoff als Malvin (Malvidin-3,5-diglucosid) anzusprechen



ist. Hierbei hat die papierchromatographische Technik ebenfalls eine gute Dienste geleistet.

Vor zwei Jahren haben wir²⁾ auf papierchromatographischem Wege gezeigt, dass das Malvin den Hauptfarbstoff der Blüten von *Lespedeza bicolor* THUNB. var *japonicum* NAKAI und von *L. floribunda* BUNGE darstellt, und ferner dass dasselbe in Form einer farblosen Vorstufe in den weissen Blüten von *L. japonica* BAILEY vorkommt. Dieser Befund hat unser reges Interesse für die Erforschung des Blütenfarbstoffs der anderen *Lespedeza*-Arten im Hinblick auf der biogenetischen Beziehung zwischen dem Anthocyanin und dessen Leukokörper geweckt.

Beschreibung der Versuche

Wenn man zur Darstellung des Anthocyanins aus Blüten frisches Material benutzen will, so ist es im allgemeinen vorteilhaft, den Farbstoff zunächst in Form seiner

* Mitteilung aus dem Nationalen Institut für Genetik, Nr. 109

** National Institute of Genetics, Mishima

1) XXV. Mittel.: Bot. Mag. Tokyo, 68, 51 (1955)

2) Hayashi, K. u. Abe, Y.: Misc. Rep. Res. Inst. Nat. Resour., 29, 4 (1953)

Bleiverbindung anzureichern, um damit die mühsame Entfernung von Wasser umgehen zu können. Bei der Aufarbeitung der *Lespedeza*-Blüten hat sich jedoch diese Methode als nur schwer anwendbar erwiesen, da das überschüssige Bleiacetat auf das Anthocyan zerstörend wirkte. In einem einzigen Fall hat es uns wohl geglückt, den Farbstoff zu gewinnen, aber die Anwendung von Bleiacetat muss in diesem Falle möglichst vermieden werden, und das Material sollte entweder mit überschüssigem Extraktionsmittel behandelt, oder sonst im Vakuum möglichst schnell entwässert werden.

a) *Isolierung des Anthocyanins über eine Bleiverbindung.* 220 g frische Blüten, die von grünen Teilen befreit waren, wurden in 450 ccm kalter, 1-proz. methanolischer Salzsäure hineingetan, und darin über Nacht gehalten. Durch Pressfiltration wurden 480 ccm roter Auszug gewonnen, zu denen unter gutem Umrühren 160 ccm 20-proz. methanolische Bleiacetatlösung hinzugefügt wurden. Der entstandene Niederschlag färbte sich hell bläulich grau und schien im grossen und ganzen aus farbarmen Begleitstoffen zu bestehen, da er durch Behandlung mit methanolischer Salzsäure in eine wasserunlösliche, rotbraune, gelatinöse Masse überging.

In die Mutterlauge, die von der obigen Bleisalzfällung befreit war, fügte man 25 ccm konz. Ammoniak in mehreren Portionen hinzu. Dabei fiel die grüne Bleiverbindung weiter aus, die sofort abgenutscht und getrocknet wurde. Ausbeute 4 g. Sie wurde fein pulverisiert, und mit 20 ccm 6-proz. methanolischer Salzsäure ins Chlorid zurückverwandelt. Beim Verdünnen der Anthocyanlösung mit gereinigtem Äther bis zur beginnenden Trübung begann das Anthocyaninchlorid sofort in Form von langen Nadeln sich auszuscheiden. Nach einigen Stunden war die Abscheidung beendet und



Fig. 1. Malvinchlorid. (\times ca. 200)

die Mutterlauge wurde beinahe farblos. Die Krystall bestanden aus abgeschnittenen, rotbraunen Nadeln mit einem Stich ins Violett (Fig. 1). In massivem Zustand sahen sie schokoladenfarbig aus und zeichneten sich durch einen grünlichen Goldglanz aus. Diese Substanz wog nur 120 mg in lufttrockenem Zustand.

b) *Isolierung des Anthocyanins ohne Anwendung von Bleiacetat.* Zur Aufarbeitung der frischen Blüten in kleinerem

Massstab hat sich folgendes Verfahren als günstig erwiesen. Zum Beispiel wurden etwa 40 g der Blüten mit 100 ccm 2-proz. methanolischer Salzsäure extrahiert, und der klar filtrierte Extrakt wurde mit reichlichem Äther gemischt. Der ausgefällte Farbstoff wurde abgesondert und mit 20 ccm 1-proz. äthanolischer Salzsäure unter Erwärmen extrahiert, wobei der Hauptanteil unlöslich zurückblieb. Aus diesem Alkoholextrakt wurde das Anthocyanin wieder mit einer grossen Menge von Äther gefällt, die Fällung in 7 ccm kaltgesättigter Pikrinsäurelösung unter gelinder Erwär-

mung aufgelöst und dann im Kühlschrank aufbewahrt. Bald trat das Pikrat als kugelförmige Nadelaggregate in Erscheinung. Die Umwandlung von Pikrat ins Chlorid wurde wie üblich durch Behandlung mit 8-proz. methanolischer Salzsäure bewerkstelligt. Nach dem Fällern mit Äther und durch Umlösen aus Salzsäure wurde das Anthocyanin rein erhalten.

Beschreibung und Charakterisierung des Anthocyanins. Das Glykosid liess sich aus 7-proz. Salzsäure sehr leicht in langen, purpurroten Nadeln abscheiden, die in massivem Zustand dunkel schokoladenfarbig aussahen, und einen schönen käfergrünen Glanz besaßen. Die lufttrockene Substanz schmolz bei 185–6° unter gelindem Aufbrausen, während sie bei recht langsamer Temperaturerhöhung von ca. 180° an zu verkohlen anging, aber unterhalb 250° nicht zum Aufbrausen kam. Die Verteilungszahl zwischen 0.5-proz. Salzsäure und *iso*Amylalkohol wurde als 1.3 gefunden; demnach verhielt sich der Farbstoff normal diglykosidisch. Wir sind dazu geneigt, ihn in die Delphinidingruppe einzureihen, da er deutlich ein für diese Gruppe charakteristisches Absorptionsband 345 m μ aufwies. Dass dieser Farbstoff methoxylhaltig ist, wurde durch seine negative Reaktion mit Eisenchlorid hingewiesen. In der Tat konnten wir nach ZEISEL-PREGL zwei Methoxylgruppen nachweisen. Die übrigen qualitativen Reaktionen sind in der unten stehenden Tabelle zusammengestellt. Allem Anschein nach ist das Anthocyanin der *Lespedeza*-Blüten mit Malvin identisch, was auch aus den folgenden Analysendaten hervorgeht.

Krystallwasser-Bestimmung:

Sbst. (lufttrocken) 21.1 mg:	Gew.-Verl. 3.6 mg (104°, P ₂ O ₅ , 4 mm Hg),
„ 4.70 „ :	„ 0.82 „ („ „).
Malvin, C ₂₉ H ₃₅ O ₁₇ ·8H ₂ O.	Ber. H ₂ O 17.25.
	Gef. „ 17.1, 17.4.

C-H-Bestimmung:

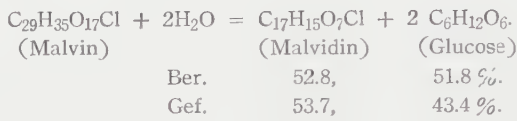
Sbst. (wasserfrei) 3.347 mg:	CO ₂ 6.195 mg, H ₂ O 1.534 mg,
„ 2.506 „ :	„ 1.294 „ „ 1.026 „ .
Malvin, C ₂₉ H ₃₅ O ₁₇ Cl.	Ber. C 50.40, H 5.07.
	Gef. „ 50.48, 50.76, „ 5.09, 4.94.

Methoxyl-Bestimmung:

Sbst. (wasserfrei) 4.23 mg:	AgJ 2.58 mg,
„ 3.10 „ :	„ 2.30 „ .
Malvin, C ₂₇ H ₂₉ O ₁₅ Cl(CCH ₃) ₂ .	Ber. OCH ₃ 8.97.
	Gef. „ 8.06, 8.94.

Quantitative Hydrolyse des Glykosids. Wegen der spärlichen Materialmenge war eine Wiederholung des Versuchs nicht möglich. Aus dem einzigen Versuch ging aber bereits hervor, dass dieses Anthocyanin zwei Moleküle von Glucose enthält. Hierbei wurden 17.5 mg von krystallisiertem Glucosid (im Vak. bei 105° üb. P₂O₅ getrockn.) durch kurzes Verkothen (3 Min.) mit 2.5 ccm 20-proz. Salzsäure hydrolysiert. Das gebildete Aglykon fiel sogleich in glänzenden Nadeln vollkommen aus, und die überstehende Flüssigkeit war praktisch farblos. Wasserfrei abgewogen, betrug die Ausbeute an zuckerfreiem Farbstoff 9.4 mg.

Die saure Mutterflüssigkeit wurde mit ein wenig Wasser verdünnt und mit einer kleinen Menge von *iso*Amylalkohol, dann mit Äther gewaschen. Die Ätherschicht hinterliess nach dem Abdampfen keinen Rückstand, sodass der ursprüngliche Farbstoff mit keiner organischen Säuren verestert war. Darauf wurde die salzsaure Zuckerlösung mit Soda bis zur schwach sauren Reaktion abgestumpft, und ohne weiteres zur Zuckerbestimmung nach BERTRAND verwendet. Zudiesem Zweck haben wir vorläufig solche Kupferoxydulmengen experimentell bestimmt, die den verschiedenen Mengen von Glucose (6.5, 7.0, 7.5 and 8.0 mg) entsprachen. Unter Benutzung dieser Vergleichsdaten wurde die Menge des vom Anthocyanin abgespaltenen Zuckers als 7.6 mg ermittelt. Demnach verläuft die hydrolytische Spaltug zahlenmässig nach der Gleichung :



Dass der Zucker Glucose ist, wurde hauptsächlich durch folgende Reaktionen bestätigt, die an Hand einer anderen Restlösung der Hydrolyse angestellt wurden. Eine Reihe von Farbenreaktionen, nämlich für Pentose nach BIAL, für Methylpentose nach ROSENTHALER, und für Ketohexose nach SELIWANOFF, fielen negativ aus. Das Zuckerosazon krystallisierte sofort und mit ganz einheitlichem Aussehen unterm Mikroskop aus der neutralisierten Hydrolyseflüssigkeit aus; es bildete in Bündel vereinigte, gelbe Nadeln, die für Glucosazon charakteristisch sind.

Zuckerfreier Farbstoff (Malvidinchlorid). Bei der Hydrolyse mittels 20-proz. Salzesäure schied sich das Aglykon in hübschen, rotbraunen Nadeln quantitativ aus.

Farbenreaktionen des *Lespedeza*-Farbstoffs

	Anthocyanin (Malvin)	Anthocyanidin (Malvidin)	Malvidin (authentisches)
Äthanolische Lösung mit FeCl ₃ (alk.)	purpurrot	violettrot	violettrot
„ NaOH aq.	unverändert	unverändert	unverändert
„ K ₂ CO ₃ aq.	azurblau	azurblau	azurblau
„ NaHCO ₃ aq.	blau	blau	blau
„ CH ₃ COONa aq.	„	„	„
„ (CH ₃ COO) ₂ Mg (alk.)	violett	blauviolett	blauviolett
„ (CH ₃ COO) ₂ Pb aq.	violett	blau (-violett)	blau (-violett)
„ AlK(SO ₄) ₂ aq.	blauer Nd.	blauer Nd.	blauer Nd.
„	unverändert	unverändert	unverändert
Fehlingsche Lösung			
in der Kälte	nicht reduz.	nicht reduz.	nicht reduz.
in der Wärme	reduziert	reduziert	reduziert
Pikrat	fadenförmig	Nadeln	Nadeln
Hauptabsorptionsbanden (in Äthanol)	540, 345 mμ.	556, 345 mμ.	556, 345 mμ.

Die Substanz liess sich am besten umkrystallisieren durch Auflösen in kleinster Menge Äthanol und Vermischen mit etwa 2/3 Volumen 10-proz. Salzsäure. Wie aus der Tabelle ersichtlich ist, stimmte das Anthocyanidin mit dem authentischen Malvidin in aller Hinsicht überein. Unterhalb 300° schmolz die Substanz nicht. Zur Analyse benutzten wir das aus der siedenden 20-proz. Salzsäure auskrystallisierte Chlorid.

Krystallwasser-Bestimmung:

Sbst. (lufttrocken) 3.3 mg: Gew.-Verl. 0.3 mg (104°, P₂O₅, 4 mm Hg).
 $C_{17}H_{15}O_7Cl \cdot 2H_2O$. Ber. H₂O 8.94. Gef. H₂O 9.1.

Methoxyl-Bestimmung:

Sbst. (wasserfrei) 4.4 mg: CH₃J 5.2 mg.
 $C_{15}H_9O_5(OCH_3)_2 Cl$. Ber. OCH₃ 16.9. Gef. OCH₃ 15.6.

Papierchromatographische Prüfung. Um das Anthocyanin weiter zu charakterisieren, haben wir die krystallisierten Präparate papierchromatographisch geprüft. Zu diesem Zwecke wurde eine Lösung von Farbstoff in 1-proz. methanolischer Salzsäure wie üblich auf einem Filterpapierstreifen aufgebracht (Tôyô, Nr. 50) und nach der aufsteigenden Methode bei 25 C mit den folgenden Lösungsmittelgemischen chromatographiert, welche wir aufs neue für die Trennung von Anthocyanfarbstoffen als sehr geeignet gefunden haben.

- | | |
|--|-------------------------------|
| (a) <i>iso</i> Amylalkohol-36% HCl-H ₂ O (5:1:1, v/v) | für Glykosid (sowie Aglykon). |
| (b) Butylalkohol-36% HCl-H ₂ O (7:2:5, v/v) | für Glykosid. |
| (c) Eisessig-36% HCl-H ₂ O (3:1:8, v/v) | für Glykosid (sowie Aglykon). |
| (d) Aceton-10% HCl (1:1, v/v) | für Aglykon. |
| (e) <i>iso</i> Propylalkohol-10% HCl (1:1, v/v) | für Aglykon. |
| (f) Eisessig-36% HCl-H ₂ O (5:1:5, v/v) | für Aglykon. |

Freilich ergab das Anthocyanin bzw. Anthocyanidin nur einen einzigen, gut definierten Fleck, dessen R_f-Werte wie folgt ermittelt wurden:

für das Glucoside:

R_f=0.03 mit (a), 0.32 mit (b), 0.61 mit (c), 0.90 mit (f), 0.39 mit (e), 0.52 mit (d);

für das Aglykon:

R_f=0.24 mit (d), 0.34 mit (e), 0.46 mit (f), 0.43 mit (a).

Die mit authentischem Präparat gemischte Probe verhielt sich ebenso. Demnach können wir darauf schliessen, dass das *Lespedeza*-Anthocyanin zweifelsohne aus Maivin besteht, und das Anthocyanidin mit Malvidin identisch ist.

Wir verdanken Herrn D. OHATA die Durchführung der Elementaranalysen und auch Herrn G. SUZUSHINO eifrige Mitwirkung bei der Sammlung und Aufarbeitung des Blumenmaterials. Der eine von uns (T. N.) wurde von dem Unterrichtsministerium finanziell unterstützt.

(National Institute of Genetics, Mishima)

ウシグソヒトヨの担子柄の細胞学的研究*

木村 勘二・武丸 恒雄**

Katsuji KIMURA and Tsuneo TAKEMARU: Meiosis in the Basidium of
Coprinus macrorrhizus Rea f. *microsporus* Hongo

1955 年 1 月 24 日受付

帽菌類の担子柄の細胞学的研究に関しては Wager^{20, 21)}, Bauch¹⁾, Kühner^{9, 10, 11)}, Bose^{2, 3)}, Ritchie^{13, 14)}, Ehrlich and McDonough⁶⁾ 外諸氏の業績があるが、我が国では僅かに若山^{22, 23)}, 後藤⁷⁾両氏の発表が見られるだけである。これらの中で *Coprinus* ヒトヨタケ属については Sass^{15, 16, 17, 18)}, 若山^{22, 23)}, Vokes¹⁹⁾, Chow⁵⁾氏等の少数の論文があるのみであり、従つて観察に疑問の点や今後に残された問題が多々ある。著者等は *Coprinus* 属のもの 1 種について、その担子柄の發育をでき得る限り順序を追つて細胞学的に観察したところを写真によつて示すと共に、既往の文献との異同を述べて見たい。

材 料 と 方 法

ウシグソヒトヨ *Coprinus macrorrhizus* Rea f. *microsporus* Hongo の複相菌糸をベトリ皿内の馬鈴薯煎汁寒天培養基に植えて 29°C で培養し、生じた子実体を供試した。本菌の子実体は始原体が現われてから急速に生長し、約 2 昼夜後には溶解し去るというように生存期間の短いものである。そのためか、子実体の各々の担子柄は皆々たい一様な發育過程を示すから、生育中の子実体が担子胞子を完成せぬ若い間にその菌傘を一部分ずつ 1~2 時間置きに切りとり、固定することにより核分裂の各時期の状態を順を追つて観察することができた。

固定液にはフォルマリン・醋酸・アルコール (50% アルコール 100cc, 市販フォルマリン 6.5cc,

氷醋酸 2.5cc の混合液) を用い、1 昼夜以上固定後、パラフィン法により 3~5 μ の切片とし、ハイデンハイン氏鉄明礬ヘマトキシリンによる染色を行つた。

観 察

若い担子柄の中には楕円形をした二つの Haploid の核が存在する。各々の核は色素に良く染まる比較的大きな 1 個と非染色性の透明な部分とからなる。Fig. 1 はこのような二つの核を持つ 1 個の若い担子柄を示す。担子柄の發育につれ 2 核は接近して癒合を始め、初期においては繭形をしているが (Karyogamy, Fig. 2), やがて 1 個の大きな楕円形の Diploid の核となる (Fig. 3~4)。この中には初め各々の核より来た二つの仁が存在するが、これも間もなく接近癒合して一つとなる。Fig. 3 は癒合しつつある二つの仁を示す。またこの核の中には糸状構造が認められるが、概してこの時期には染りが弱い。

減数第一分裂が始まると先ず染色性の Leptotene chromosome が核内に現われ (Fig. 5), それらが互いに平行に対合の状態 (Zygotene stage, Fig. 6) を示した後核の一隅に集まる (Synapsis, Fig. 7)。間もなく染色体は核内に拡がりながらだんだん肥厚し (Pachytene stage, Fig. 8) Diakinesis (Fig. 9~11) の時期に入り、各染色体は非常に短縮していつて 4 個の短い二価染色体となる。次いで核膜は不明瞭となり仁は消失するが、時としては Anaphase に至るまで仁の残ることもある (Fig. 29~30)。二価染色体は更に短縮して遂には 4 個の粒状の染色体となつて赤道板に並ぶ (Metaphase I, Fig. 12)。なお、この際に紡錘体が現われ両極に中心体が明瞭に観察される (Fig. 13)。Anaphase I に入ると各々の二価染色

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体は一個染色体に分れて両極に引き裂かれていく (Fig. 14), Fig. 15 は粒状の 8 個の一個染色体が紡錘糸の上に乗っているのを示す。Late anaphase I (Fig. 16) から Telophase I (Fig. 17) にかけて一個染色体が両極に集まり、二個染色体集団を結ぶ紡錘糸が見られる。また、時としてこの際両極より発した放射状の構造が見られることもあった (Fig. 18~19)。次に核は Interphase (Fig. 20) を経て減数第二分裂の Metaphase に入り、一個染色体が四つずつ各々の赤道板に並ぶ (Fig. 21)。この頃になるとそれぞれの核の中の両方に中心体が現われ、紡錘糸により各染色体は縦裂して両極に引き裂かれていくが、二つの分裂軸はほぼ平行である (Anaphase II, Fig. 22)。そして Telophase II においては第一分裂の場合と同様に両染色体集団を結ぶ紡錘糸が観察される (Fig. 23)。以上の経過の後に担子柄の中に 4 個の Haploid の核ができあがる (Fig. 24)。

次に第一分裂の Late prophase 及び Metaphase で Fig. 25~28 に示すような染色体 8 個を有する担子柄も見られた。これらの担子柄は上述の場合に見られたような粒状でなくて長短の別があり、4 対の相同染色体らしいものを区別することができた (特に Fig. 27)。また、第一分裂の Anaphase で 16 個の棒状の染色体を持った担子柄 (Fig. 29~30) 及びこれの側面観と思われる担子柄 (Fig. 31) も観察されたし、第二分裂の Prophase で Synapsis と思われる 2 個の核を有する担子柄 (Fig. 32) も見られた。これらは染色体の数や形並びに Synapsis の時期の点で前に述べた前還元の場合とは多少その様子を異にするものであった。

考 察

若山^{23,25)} は *Coprinus micaceus*, *C. atramentarius* その他多数の茸菌類の種について細胞学的研究を行つたが、これらの担子柄における減数分裂はすべて前還元であると断じている。また Vokes¹⁹⁾ の *Coprinus atramentarius*, 後藤⁷⁾ の *Sclerotium Rolfsii*, Ehrlich and McDonough⁶⁾ の *Schizophyllum commune* の細胞学的研究においてもその発表されたところは前還元の場合だけであり、後還元の場合については触れていない。

一方 Hanna⁸⁾ は四極性の *Coprinus lagopus*

の個々の担子柄に生じた 4 個の胞子の交配型分析を行い、互いに交配型を異にする 4 種類の胞子を持った担子柄も存在するのが認められたことから、同菌の担子柄における減数分裂は後還元であると報告している。また Newton¹²⁾ は同菌で, Brunswik⁴⁾ は四極性の *Coprinus fimetarius* で, Hanna と同じ実験を行つたが、両氏は 4 種類の胞子を持つ担子柄の出現率から推して減数分裂は前還元でも後還元でもなく、或る相同染色体は第一分裂で分離し他の相同染色体は第二分裂で分離するものであろうと述べている。

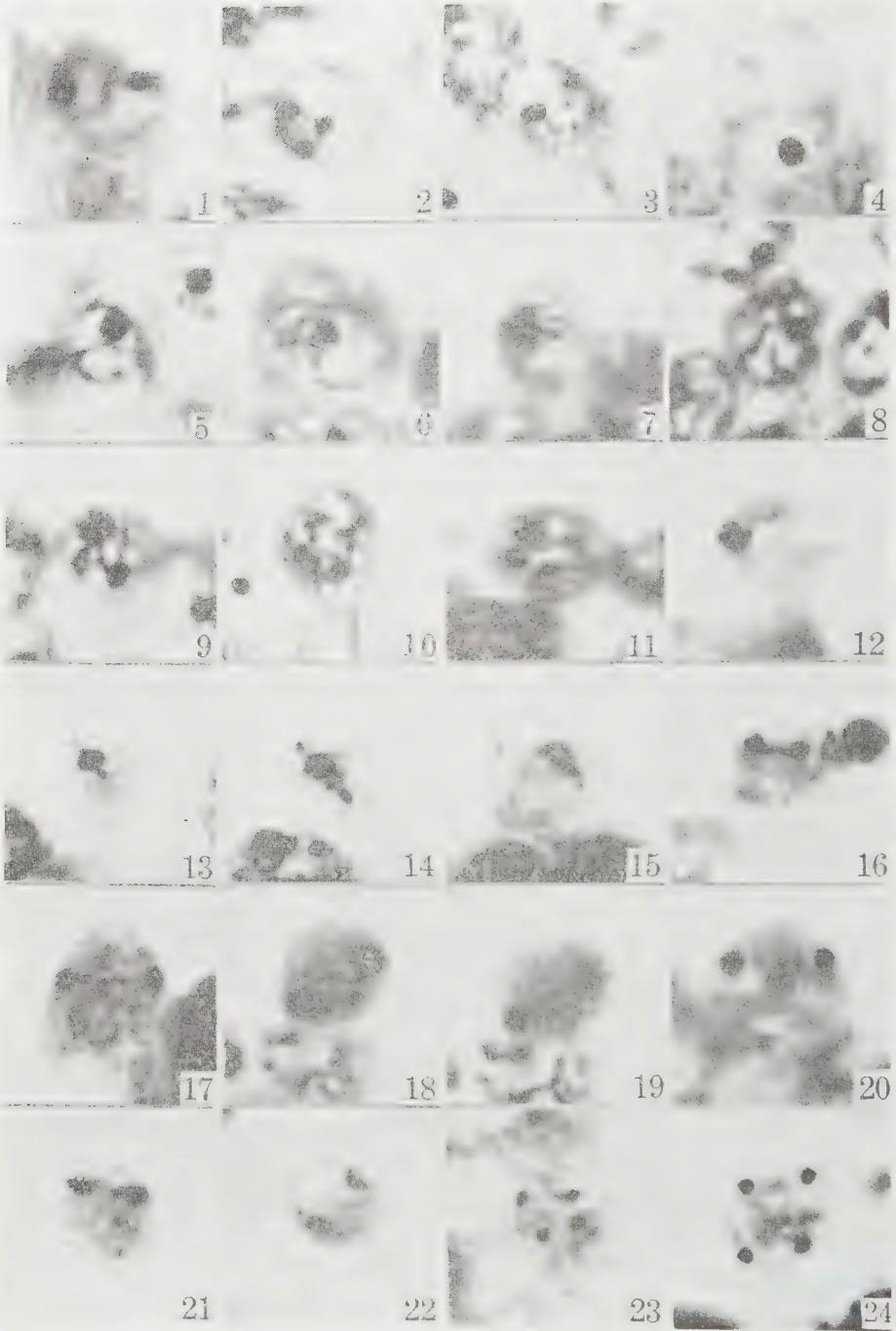
著者等の観察の結果は Fig. 1~24 に示すように前還元の場合も見られたが、一方 Fig. 27~28 のように第一分裂の Metaphase で前還元の場合の粒状の 4 個の二個染色体とは大いに趣きを異にする棒状乃至棒状の 8 個の染色体を有する担子柄も観察された。これらは染色体の数及び形の上から考えて 4 対の相同一個染色体が赤道板に並んだものと見たい。すなわち前者の粒状が Meiotic figure であるのに対して、この場合は Mitotic figure であると考えられる。また Fig. 29~30 に示される第一分裂の Early anaphase における 16 個の染色体は上記の 8 個の一個染色体が縦裂して両極に移動しつつあるもののように思われる。これらの観点に加うるに第二分裂の Prophase で Synapsis と見られる 2 個の核を持った担子柄 (Fig. 32) も観察されることから、著者等は同菌の担子柄内の減数分裂は前還元の場合も後還元の場合もあるものと考えたい。

なお Fig. 9 に示すように、第一分裂の Diakinesis において二個染色体が 3 個、一個染色体が 1 対存在する像も見られたが、これが Newton, Brunswik 等の説いているところをうらづけするものであるかどうかは今後研究を続けた後に触れることにする。

最後に本研究について有益な御助言を賜つた東京大学和田文吾博士並びに岡山大学猪野俊平博士に深く感謝する。

摘 要

1. ウシグソヒトヨ *Coprinus macrorrhizus* Rea f. *microsporus* Hongo の若い担子柄の中には初め Haploid の 2 核が存在するが、これらが癒合した後減数分裂が行われ 4 個の Haploid の核が



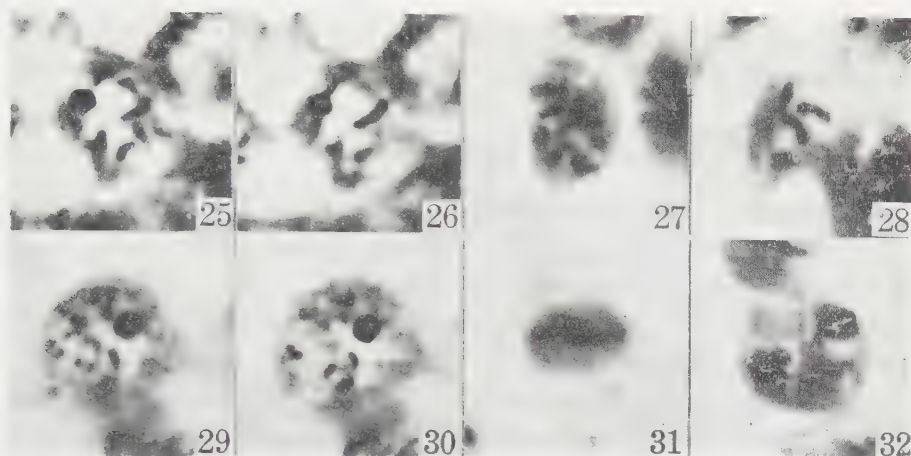


Fig. 1~24. The meiosis in the basidia of *Coprinus macrorhizus* Rea f. *microsporus* Hongo. All magnifications are $\times 2600$. All microphotographs are unretouched.—Fig. 1. Two haploid nuclei in young basidium.—Fig. 2. Fusion of two haploid nuclei (karyogamy).—Fig. 3. Diploid nucleus, in which two nucleoli are fusing.—Fig. 4. Resting stage.—Fig. 5. Leptotene stage.—Fig. 6. Zygotene stage. Four pairs of chromosome threads are visible.—Fig. 7. Synapsis.—Fig. 8. Pachytene stage.—Fig. 9. Early diakinesis. A pair of chromosome threads is attached to the nucleolus. Three other bivalents are free at 9, 11 and 12 o'clock.—Fig. 10. Four bivalents at mid-diakinesis, one of which is attached to the nucleolus at 8 o'clock.—Fig. 11. Four bivalents at late diakinesis. The nucleolus is at 8 o'clock.—Fig. 12. Metaphase I (polar view). A group of all four bivalents is at 9 o'clock.—Fig. 13. Metaphase I (side view). One bivalent is lost in a mass of other three chromosomes.—Fig. 14. Early anaphase I. Centrosomes are clearly visible at both poles.—Fig. 15. Mid-anaphase I. Eight dyads on the spindle are visible, some of them overlapping.—Fig. 16. Basidium at late anaphase I. Spindle connecting sister chromosome groups is clearly stained.—Fig. 17. Telophase I. Spindle is slightly stained.—Fig. 18~19. Two focal levels of the same telophase I basidium. Notice a radial structure from each pole.—Fig. 20. Two sister nuclei in interphase.—Fig. 21. Metaphase II (polar view). Two nuclear plates, right one slightly out of focus, are visible. Four chromosomes are recognizable on the left nuclear plate. Fig. 22. Anaphase II (side view). A centrosome is visible at each pole.—Fig. 23. Telophase II. Spindles are slightly stained.—Fig. 24. Four nuclei resulting from the two successive divisions.

Fig. 25~32. Microphotographs suggesting the possibility of post-reduction. All magnifications are $\times 2600$. All photographs are unretouched.—Fig. 25~26. Two focal levels of the same late prophase I nucleus. All eight univalents, some of them slightly out of focus, are visible in fig. 25. Two are in focus in fig. 26.—Fig. 27~28. Metaphase I. All eight univalents are visible in fig. 27. In fig. 28, four univalents are observed, the remainder four chromosomes being cut off.—Fig. 29~30. Two focal levels of the same early anaphase I nucleus (polar view). Sixteen monads are visible, some of them overlapping. Nucleolus is yet remaining.—Fig. 31. The side view of same stage. Notice the rod-shaped chromosomes. Centrosomes are clearly visible at both poles.—Fig. 32. Two sister nuclei, each of which is at synapsis.

形成される。

2. 減数第一、第二分裂共に紡錘体の両極に中心体が見られる。

3. 減数分裂は前還元の場合も後還元の場合もある。

4. 本菌の染色体数は $n=4$, $2n=8$ である。

Summary

1. The young basidium of *Coprinus macrorrhizus* Rea f. *microsporus* Hongo is binucleate in the primary stage. After the fusion of these two primary nuclei, the meiosis follows, and gives rise to four daughter nuclei.

2. At each pole of the spindle, a centrosome can be seen in both the first and the second meiotic divisions.

3. Cytological evidence is presented that the reduction of chromosome number takes place either at the first (prereduction) or the second division (post-reduction) of the meiosis.

4. The haploid number of chromosomes for this fungus is observed to be 4, and the diploid number of chromosomes is 8.

5. Microphotographs substantiating the description are presented.

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本 会 記 事

役 員 の 移 動

会 長 選 挙

昨年の総会でできまつたとおり会長の改選が3月行われた。3月16日全会員826名(2月末日現在の)に投票用紙と案内を発送し、直接学会連系係あての郵便による投票を求めた。単記無記名、4月5日(到着)しめきり。なお今年2月発行の会員名簿にのつている方でも2月末日までに29年分の会費(30年になつて入会した方はよいが)を納めなかつた方は失格として投票用紙を送りなかつた。

会則の付則第3第1条に会長選挙の際評議員会は若干名の候補者を推薦することができるという規則がある。それによつて評議員会は朝比奈泰彦、篠遠喜人、田宮博、服部静夫、本田正次の5氏(アイウエハ順)を推薦した。(現会長小倉謙氏も候補者として話題にのぼつたが、この次こそどうしてもやめたいといふ前々からの同氏の切なる希望により今回は推薦することを断念した)

4月6日開票、次のとおりの結果となり、最高

服 部	静 夫	88
本 田	正 次	65
篠 遠	喜 人	53
朝 比 奈 泰 彦		47
田 宮	博	28
そ の 他	8 氏	16
合 計 投 票 数		297

点者服部氏は同日会長たることを受諾され、引きつがれた。

新会長 服部静夫氏

なお前会長小倉謙氏は昭和21年4月3日就任以来満9カ年の長い間(その間6回の改選に当選)引続き会長として在任され戦後の本会興隆のため多大な力をつくされた。ここに同氏に対して感謝すると共に新会長を迎えて本会のますます発展することを祈る次第である。

評 議 員 選 挙

これも昨年の総会でできまつたとおり3月改選された。会則の付則第3第2条に従つて行われたわけで、各支部毎に支部会員によつて選出された評議員の氏名が4月5日までに本部に報告されたがその結果は次のとおりである。なお定員は前記の条文にあるように各支部に属する会員数(有権者たる条件は会長の場合と同様)によつてきまるが、選出の仕方は各支部の自由であつた。また、評議員は引続き3期選出されることはできないという条文は今回選出の方からはじめて適用されることもち論である。

北海道支部(定員2名) 山田幸男、松浦 一
東北支部(定員2名) 木村有香、長尾昌之
関東支部(定員6名) 前川文夫、原 寛、津山尚、三輪知雄、今関六也、亙理俊次
北陸支部(定員2名) 正宗巖敬、柴田万年
中部支部(定員2名) 熊沢正夫、森 健志
近畿支部(定員3名) 芦田譲治、新家浪雄、今川駿一郎

中国四国支部(定員3名) 下斗米直昌、堀川芳雄、猪野俊平
九州支部(定員2名) 瀬川宗吉、小島 均
以上合計 22 名

幹 事 長 お よ び 幹 事 の 交 代、新 任

幹事長伊藤洋氏に代つて門司正三氏が、編集幹事のうち山本茂氏に代つて高野伸二氏がそれぞれ就任された(4月から)。

また庶務幹事として原襄氏が増加された(4月から)。図書幹事として古沢潔夫氏があらたに加わつた(1月から、これは本会ほか3学会と文部省との協力で毎年編集発行されている生物学抄録の総まとめ、採録、校正などを仕事とするもので今までは毎年どなたかにお願ひしたり幹事長がやつたりしていたのであるが、仕事が恒常的であり相当負担がかかるので専門の幹事を置くことにしたものの)。

会 員 の 移 動

新 入 会 (3 月分, カツコの中は所属支部)

伊藤三郎 (関東) 昭和葉大・東京都大田区調布鶴
ノ木町 128 桶川方伊藤 弘 (東北) 秋田県立花輪高校・秋田県鹿角
郡花輪町新田町小島俊郎 (関東) 山梨県富士吉田市上吉田町山梨
県林業試験場藤田幹雄, 門田孝子, 小竹 章, 進藤公夫, 山本
正明 以上 5 名 (中国四国) 広島大理植斎藤全生 (中部) 静岡大農・静岡県磐田市河原町
4055-3天野良之 (中部) 静岡大文理生物・静岡市長谷町
13

木村孝一 (関東) 東大理植

任 所 変 更 (3 月分)

高橋大蔵 (中国四国) 愛媛県温泉郡南吉井村南野
田

小松崎弘 (関東) 茨城県北相馬郡菅生村

坂村 徹 (中国四国) 広島市古田町古江 1370

会 員 名 簿 正 誤

3 頁左 14 人目柳沢勉 (東北支部) を 9 頁左 19
人目 (北陸支部) に入れる

本会名誉会員 E. B. Babcock 氏 (カリフォルニア
大学名誉教授) は昨年 12 月 8 日 77 才の高齢で死去
されました。

ここに報告し謹んで哀悼の意を表します。

日本植物学会

支 部 通 信

関 東 支 部

1 月例会。29 日東大理植物学教室において。講
演 (1) 島地謙: カシ属植物の木材解剖学的性質
とその類縁について (2) 柴田承二: 菌類の色素
について。

2 月例会。19 日東大。講演 (1) 湯浅明: 遺伝
における色素体の行動 (2) 飯塚広: 黄変米につ
いて。

支部大会。4 月 17 日林業試験場において。講
演 (1) 小山鉄夫: スゲ属の多元的系統について
(2) 鳥山英雄: オジギソウの葉枕の細胞生理学的
研究 V (3) 新崎盛敏: 褐藻植物の系統と分類 (4)
渡辺篤: 空中窒素固定能を有する藍藻の稲の收穫

に及ぼす影響 II (5) 荒野久雄: 菊科ハハコグサ
亜類などの核型分析と系統関係考察 (6) 保井コ
ノ: トウモロコシの栄養器官及び生殖器官特に雌
花穂の発生機構について (7) 柴田治: サンショ
ウモの日長反応 (8) 鈴木貞雄: 栃木県における
暖帯林の分布と構成 (9) 佐藤七郎: 組織による
ヤヌスグリーン B 還元とミトコンドリア染色の
機構について (10) 佐藤七郎・木村孝一: 幼胚植物
のホモジエネートにおける T T C 還元系の諸特
性, 特に生体切片との比較について (11) 百瀬静
男: シダ類の前葉体における造精器の位置につ
いて (12) 亘理俊次: 北九州の松岩の樹種につ
いて。特別講演 館脇操: スカンジナヴィア植物紀
行。

第 20 回 日 本 植 物 学 会 大 会 予 告

昭和 30 年度の大会は中国四国支部でお引受けいたし, 広島市で下記のとおり開催することに決定しま
した。詳細は追つてご通知申し上げます。次号 (5 月号) に講演申込用紙等を挿入する予定であります。

期 日 昭和 30 年 10 月 12 日-14 日
場 所 広島大学理学部

(中国四国支部)

On the Excitation Phenomena in Embryonic Plants of *Vigna sesquipedalis* Caused by Electric Stimuli, and Presence of Polarities Concerning the Excitability

by Hisashi OKAMOTO*

岡本 尚: ミトリサヘゲ幼植物体の電気刺激感応現象と極性の存在について

Received January 27, 1955

During the works on the measurement of electric potential distribution in *Vigna* embryo, the influence of light as a disturbing factor for the resting potential was confirmed besides the influence of mechanical stimulus (1). Similar phenomena have been reported, so far as yet known, by Yendo on other plant materials (2). Temporary falls of the potential were observed at the alteration of light- and dark-phase and vice versa, meaning the generation of the action potential.

Prior to further investigations on this phenomenon, two problems must be resolved: (1) to observe whether such fall of the potential represents a general excitation phenomenon which can be caused by stimuli of some other nature, for instance, electric or thermal agitation, and (2) to determine the quantitative relations assumed to be established between stimulation and excitation.

In the present paper, results of the experiments about electric stimulations on hypocotyl of embryonic plants of *Vigna sesquipedalis* are reported.

Methods

Embryos of *Vigna sesquipedalis* which had been cultured in washed sand two to six days long in the dark at 30°C were employed as materials. Rectangular current pulses (1—60 volts) were applied to the hypocotyl by means of a constant current stimulator of ordinary type. The arrangement of electrodes attached to the seedling was as follows; one of the measuring electrodes (M^+) was placed in the middle of two stimulating ones (S^+ & S^-), except when polarity in the plant body was further precisely investigated. Another measuring electrode (M_0) was always fixed on the boundary of hypocotyl and radicle; thus between the measuring electrodes, in unexcited states, the resting potential difference was set up (see Fig. 1).

The stimulating electrodes** were at a distance of ca. 16 mm apart from each other and the resistance between them was normally evaluated as 100 to 300 Kiloohms

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** Ordinary Zn/ZnSO₄ electrodes

(which varies with excitation), while the resistance of electrodes themselves was ca. 30 Kiloohms. Thus the maximum intensity of the stimulating current was 0.6 mA, its maximum efficiency 0.036 Watt.

Extents of the potential change were directly read from amounts of deflection of the galvanometer in the electrometer circuit (1) calibrated beforehand.

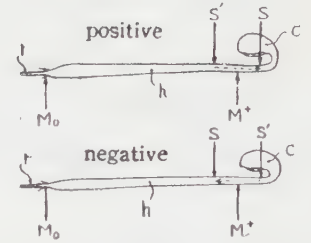


Fig. 1 Ordinary arrangement of the electrodes

Results

1. Presence of the Polarity.

A stimulus of 60 volts in strength immediately caused a remarkable fall of the resting potential at the stimulated part, which was also accompanied with a lowering of the ohmic resistance (Fig. 2 & 3).

The lowered potential recovered to its normal value within 60 to 120 minutes. After the complete restoration of the resting potential, a repeated stimulus gave the same effect (Fig. 4). It is interesting that the stimulus with a current of reversed direction gave rise to a different type of excitation, as shown in Fig. 2 & 3. Denoting one direction of the elongation of hypocotyl as "positive" and the reversed one as "negative", a stimulus of the positive direction caused a relatively deeper lowering of potential and its restoration occurred sharply. On the other hand "negative" stimulus of the same strength and duration caused a distinctly shallow fall of potential, which often required a few minutes to attain its maximum value and the restoration of the resting potential occurred slowly. I distinguish the excitation observed in the former case as "type I" from "type II" in the latter. The type II is not a subsequent effect of the type I (Fig. 3).

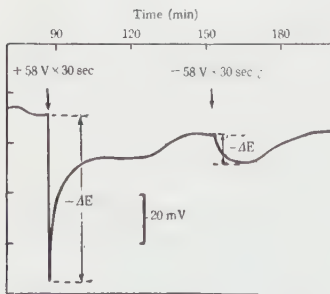


Fig. 2 Potential changes at the measuring electrode (M^+) caused by the electrical stimuli of mutually opposite directions.

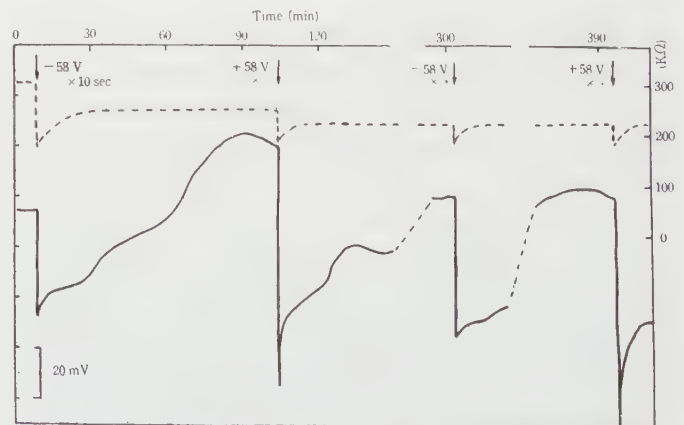


Fig. 3 The same as Fig. 2. Changes of the ohmic resistance (dotted line) between two stimulating electrodes are also illustrated.

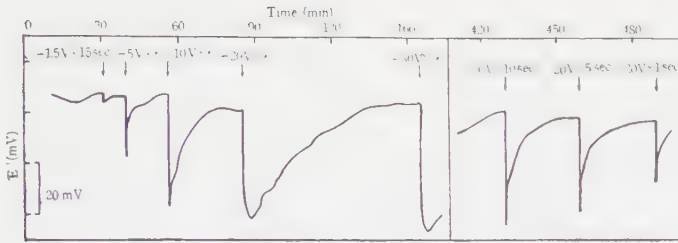


Fig. 4 Potential changes by the repeated stimuli of various magnitude.

2. Quantitative Relations between Excitation and Stimulation.

As a measure of the excitation, we can adopt the magnitude of a fall of the resting potential ΔE (in millivolts). As for the stimulus, two quantitative factors must be taken into consideration, namely the intensity S (in volts) and the time of duration t (in seconds).

a) Stimuli of 2.5 to 60 volts in strength and 1 to 40 seconds in duration were applied to hypocotyls of 2 days old embryos. Some typical results are shown in Fig. 4. The relations between values of the responses and applied stimuli are summerized in Fig. 5. It is clearly perceived that the effect of stimulus takes a constant level above 10 sec. of duration corresponding to a given strength of the electric impulse in every cases and larger excitation cannot be obtained unless more intense

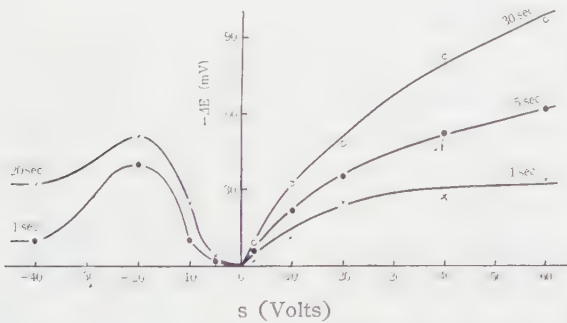


Fig. 6 Relation between maximum fall of the potential (ΔE) and intensity of stimulus (s) observed in the embryo of 48 hrs. culture.

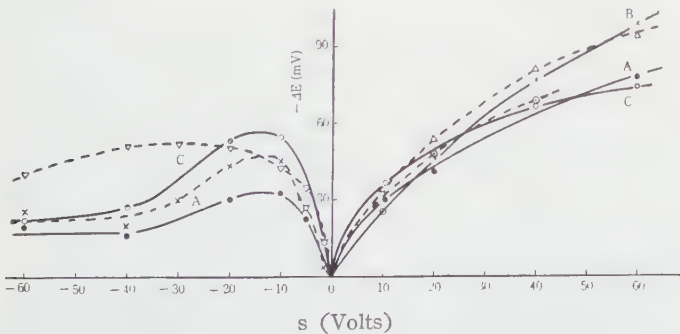


Fig. 7 Relation between maximum fall of the potential and intensity of stimuli observed in the embryo of 96 hrs. (continuous line) and 144 hrs. (dotted line) culture, ($t=15$ sec).

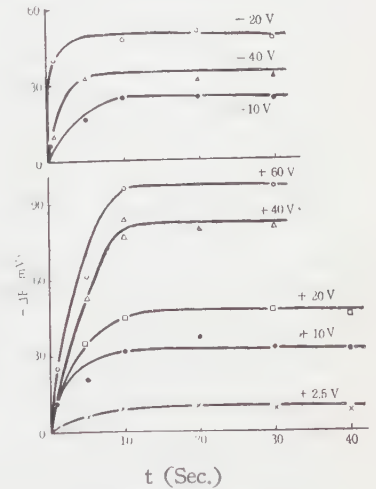


Fig. 5 Relation between maximum fall of the potential (ΔE) and duration of stimulus (t) observed in the embryo of 48 hours culture.

stimulus is applied. The results upon the intensity-excitation relation are also represented in Fig. 6 in regard to the direction of stimuli, where the type of responses is shown to vary in the form of curves apparently concerning the direction of the stimulating current.

So far as the duration (t) has a value less than 10 sec. and concerning the positive stimuli, relation between stimulation products P (intensity \times duration; volts \times sec.) and the intensity of responses can be described approximately by an empirical equation approximately:

$$-\Delta E = P^{0.74}$$

b) The stimulus-excitation relation was investigated with 4 and 6 days old embryos, keeping the duration of stimuli constant (15 sec.) in this case.

The curves illustrated in Fig. 7 were asymmetrical concerning both sides of the abscissa, confirming the aforementioned fact. So far as stimuli were applied in the range of 1-30 mm apart from the cotyledon, essentially the same relation could be observed irrespective of the age of plants.

3. Changes of Electric Potential Distribution with Local Excitation.

As mentioned above, there exists a "polarity" along the growth axis of hypocotyl in regard to the excitability. Then it may come into question whether the intrinsic origin of this phenomenon is of a structural nature or otherwise the polarity depends simply upon the polarization of the cell surface caused by the electric stress at the contact point of the electrodes. In order to resolve this problem, relations between the excitation and the resulted changes of electric potential distribution were investigated. Some typical examples of these determined relations are shown in Fig. 8.

The results are summarized as follows:

a) Changes of the potential distribution caused by the "positive" stimuli differ markedly from those caused by the "negative" ones. This indicates the occurrence of an alteration of polarization, or entrance and departure of ions at the contact points.

b) Fig. 8 shows a feature of transmission of excitation along the growth axis and slow recovery of the normal potential distribution. The velocity of transmission calculated from the time spent from the application of a stimulus till the arrival of the maximum excitation at a certain point is 0.2-0.5 mm per minute.

c) So long as the arrangement of electrodes described in the paragraph of

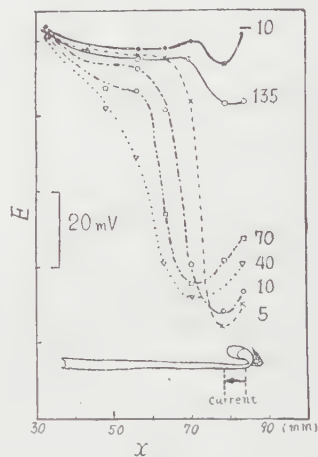


Fig. 8 Changes of the potential distribution with the excitation caused by a negative stimuli (-54V), transmission of the excitation along the growth axis of hypocotyl and its recovery process. Each curve represents potential distribution at the stage which is given in minutes at the right hand of the curve. The stimulus was given at time 0.

methods are adopted, types of the relation between stimulus intensity and excitation are determined exclusively by the direction of stimulating current, and do not depend upon the inclination of initial gradient of the resting potential on the stimulated part ($\frac{\partial E}{\partial x} > 0$ or $\frac{\partial E}{\partial x} < 0$, where x is the coordinate along the elongation axis).

4. Polarity in the Radial Direction of Hypocotyl.

Concerning the above mentioned indication 3.-a) further exploration should be carried out upon the existence of the radial polarization. In the experiments hitherto executed, one of the measuring electrodes M^+ was always set in the middle of two stimulating ones in order to avoid possible appearance of the effect of so-called "law of polar excitation". Nevertheless, polarity was observed in a difference of the electrical responses according to the direction of stimulating current.

Now, if one of the measuring electrodes (M^+) be put upon the very position of either of the stimulating electrodes, four different ways in the flow of the stimulating current must be taken into consideration at this portion. Those are the possible combination of the following two factors.

The acropetal current along the axis of hypocotyl be expressed by $A+$ and the basipetal current by $A-$. The current which flows out of the stem be denoted by $R+$ and the one that flows in by $R-$ (see Fig. 9).

If there really exists a polarity in radial direction of hypocotyl, electric responses of the type shown in Table 1-a will be expected to occur for each arrangement of the electrodes considered. On the other hand, supposing only one kind of polarity along the growth axis, the expected type of the excitation must offer another figure shown in Table 1-b.

Experimental results shown in Fig. 10 and Table 2 indicate that the relations

Table 1-a

		R	
		+	-
A	+	type I	type II
	-	type I	type II

Table 1-b

		R	
		+	-
A	+	type I	type I
	-	type II	type II

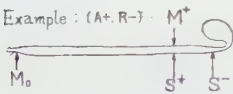


Fig. 9 An example of the arrangement of the electrodes for the investigation of the polarity, providing one of the four different ways in the flow of stimulating current, ($A+$, $R-$).

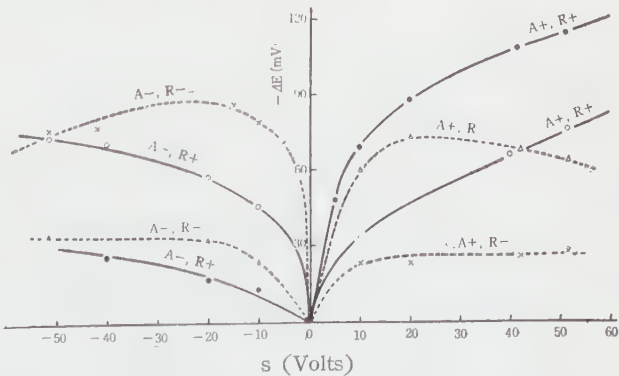


Fig. 10 Electric responses caused by the four different types of stimulating current.

represented in Table 1-a are proper. It is concluded from this that a polarity in the radial direction does actually exist.

This fact, however, does not mean to exclude the presence of polarity along the elongation axis, for if the latter might be absent, the stimulation-excitation relation would become symmetrical concerning the abscissa and any difference in excitation type could in no way be realized, when one of the measuring electrodes (M^+) was put in the middle of the two stimulating ones as in the previous procedure. The "neutral point" where contradictory influences brought about by the two stimulating electrodes are just cancelled out (and accordingly one should expect symmetrical responses on the measuring electrodes concerning the abscissa of impulse strength, is fairly biased to the root side. In this meaning, the polarity along the elongation axis must also be recognized to exist.

Table 2. $-\Delta E-S$ relations with 3 days old hypocotyls, $-\Delta E$: values of potential fall (mV).

Exp.		1	2	3	4	5	6	7	8	9
Mode of stimulation	A	+	+	+	+	-	-	-	-	-
	R	+	+	-	-	+	+	-	-	-
	$\frac{\partial E}{\partial E}$	+	-	+	-	+	-	+	-	-
S (Volts)	5	48.5						71		
	10	69	33	60	23	46	13	79	24	46.5
	20	88		73	23	58	17	86	33	
	40		66			71	26.5			
	44	108		68	26			77.5		
	53	114	76	64	28	74		76	34.5	46.5
type		I	I	II	II	I	I	II	II	II

Discussion

Compared with the electric stimulation, the light stimulation requires a lag time of 1-10 minutes until the action potential is established. Such a lag is entirely absent in the former case. Some sequent reactions may participate in the light stimulation process from reception to excitation. The process of excitation is very slow in both cases in comparison with that of animal tissues, It requires 10-200 minutes until complete recovery of the initial resting state is attained, while the transmission velocity of excitation is also about 10,000 times slower than that of neural organs. Histological and protoplasmic structures of plant organs seem to be very primitive, or extremely low differentiated so far as the function of excitation and transmission are concerned.

The threshlod value of the electric stimulus is presumably accounted very small (which has not yet been determined).

Provided that the intensity of stimulus is not too small, for instance above 1 volt, the excitation takes place complying with Weber-Fechner's law, except cases

with "negative stimuli," where the relation appears to be somewhat complicated. In Fig. 11, $-\Delta E$ values are plotted against $\log S$ and $\log \left(1 + \frac{S}{5}\right)$ respectively.

According to the so-called "law of polar excitation" found in neural and muscular tissues, an excitation may occur primarily at the negative electrode when stimulating current begins to flow, and no anodal excitation can take place until the current ceases. The polarization at both contact points takes reversed orientation to each other, namely depressing the excitability at the anode and raising it at the cathode. Though the rule also seems to hold in this case, hypocotyl is a multicellular organ and direct validity of this law is still in question, because the excitation occurs immediately after the stimulation despite of its slow transmission velocity, at the position between and fairly apart from the two stimulating electrodes, where cells should be subjected to both cathodal and anodal excitation.

It would require further extensive investigations to ascertain whether the polarity along the elongation axis may be of a structural nature or else may depend upon a certain kind of inclination of cell excitability. This inclination of cell excitability may diminish towards the root tip and cause less effective influence of the root side impulse upon the geometrical midpoint of the two stimulating electrodes. The latter interpretation seems to be more probable, if we examine the results shown in Table 2 precisely.

It is an accepted fact that when a neural fibre is excited, a rapid entrance of Na^+ ions and rather slow departure of K^+ ions are recognized with an abrupt vanishing of the resting potential.^{3, 4, 5} This means a reversible abolishing of the unbalanced ionic distribution preserved by the plasmamembranous selective permeability. Assuming a similar mechanism for the plant tissues, the polarity of the radial direction can be interpreted as a resultant effect of two factors, namely the transference of ions which must have opposite directions at both contact points and an excitation process of a more general nature which should bring about the same lowering of potential at every place. These two factors would be formulated in such manners as follows.

In the first place, there must be an ionic shift distorted by the applied potential

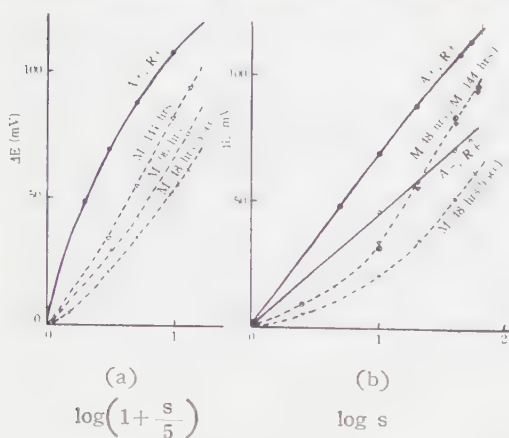


Fig. 11

- a) Relation between $-\Delta E$ and $\log \left(1 + \frac{S}{5}\right)$
 b) Relation between $-\Delta E$ and $\log s$.

$\propto \frac{RT}{F} \log \left(\frac{C_0}{C_0 - KS} \right)$, where C_0 is the initial concentration of cation, S the inten-

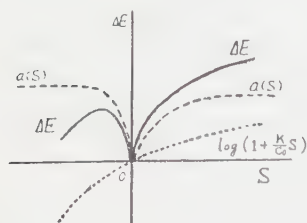


Fig. 12 Schematic illustration of the resultant excitation produced by the linear combination of two factors.

sity of stimulus and K a constant which refers to the length of stimulating time. Assuming $KS \ll C_0$, it will become $\frac{RT}{F} \log \left(1 + \frac{K}{C_0} S \right)$. As the second factor we will represented the excitation process caused by the abolishing of the resting ionic distribution by $a(S)$. Then the resultant excitation can be written in the form of a linear combination of the two factors as the first approximation for a certain limited range of S .

$$-\Delta E \propto \frac{RT}{F} \log \left(1 + \frac{K}{C_0} S \right) + a(S)$$

The relation is illustrated in Fig. 12 schematically. The asymmetrical form of $a(S)$ was traced in order to consist more closely with the experimental results observed, in which $\left(\frac{\partial E}{\partial S} \right)_{S=0}$ in the negative side of S is larger than that in the positive side.

I wish to express my gratitude to Professor T. Mori for his constant advice during this work. Thanks are also due to Mr. I. Watanabe (Central Research Institute of Electric Power Industry) for his kind aid in constructing apparatus.

Summary

(1) Temporary falls of the resting potential (maximum c. a. 100 mV) observed in the seed embryo of *Vigna sesquipedalis* are supposed to belong to the category of the general excitation phenomenon, because they can be brought about by stimuli of various nature in the some manner, although their processes are some 10^3 — 10^4 times as slow as those in animal tissues.

(2) Applying rectangular current pulses (1—60V) on the hypocotyl of the plant by means of two accessed stimulating electrodes, the presence of a polarity in parallel with the growth axis was confirmed concerning the excitability; fairly distinguishable two types of the electric responses (I & II) were detected, each corresponding to the positive (acropetal) and negative (basipetal) direction of the stimulating current applied. In this case one of the measuring electrodes (M^+) was placed in the middle of the two stimulating electrodes and another one (M_0) on the boundary of hypocotyl and radicle as counter electrode.

(3) A double propagation of excitation with velocity of 0.2—0.5 mm per minute along the growth axis was also observed.

(4) Quantitative relations between intensity as well as length of stimulus and excitation were investigated. Excitation of the type I takes place complying with

the Weber-Fechner's law, while that of the type II has its maximum at a certain strength of stimuli.

(5) Further experiments, where M^+ was placed on the same position as either of the stimulating electrodes, proved the existence of one more polarity in the radial direction of hypocotyl, namely, out flux of the stimulating current from the surface of the organ causes the excitation of the type I while influx of the stimulating current causes the excitation of the type II, irrespective of the direction of current along the growth axis.

(6) Thus the polarity along the growth axis reveals itself in the deviation of the "neutral point" towards root tip concerning the excitation types. The nature of the radial polarity was discussed on the basis of the movement of ions through stressed protoplasmic membranes.

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Effect of the Short Day Treatment on the Growth Periodical Variation of Osmotic Value in Soybean Plants*

by Shosuke KAKU**

賀来章輔: ダイズの生育週期的滲透価変化に及ぼす短日処理の影響

Received February 21, 1955

The effect of short day treatment on flowering was already studied by many investigators. According to Sō et al⁶⁾ the increase of cell sap concentration and reducing sugar was observed in a plant of short day nature when it was laid under short day photoperiod. The present author took up the problem concerning the influences of short day treatment on osmotic values, as no report could be found on this question. In the previous paper³⁾ he stated that the variation of osmotic value of a plant was induced firstly by growth periodicity and secondarily by climatic factors. The short day treatment accelerates the growth period of a short day plant and the plant become to grow in unnatural seasonal conditions. So that the studies of a short day treated plant will also be valuable for solving the problem of the periodical variation of osmotic value of a plant.

Material and Method

Soybean (*Glycine hispida* Max.) of a late season variety "Kyushu Autum No. 2" were sown on May 14, 1953. The seedlings were planted in pots. The soil moisture in the pots was kept at 80 % of the saturated water capacity. The experimental plots were divided into three as follows; (A) Control plot of natural day-length, (B) Late treated short day plot, the treatment was commenced on July 5, 43rd day after germination, (C) Early treated short day plot, the treatment was commenced on June 1, 8th day after germination. The treatment was carried out as follows: the daily light period was shortened to 10 hours before podding. The osmotic value of upper epidermal cells of leaves was measured at incipient plasmolysis in KNO₃ solution. The values are the means of five leaves representing vertical position of the plant.

Experimental Results

(1) **Shortening effect on growth periods** The flowering period arrived in (B) 15 days and in (C) 39 days earlier than the control (A) (Table 1). In (B) and (C)

* Problem of physical and physiological dryness. Rep. 17 by Y. Fukuda;

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podding began only 4~5 days after the commencement of flowering, while in control (A) it took place three weeks after. The short day treatment accelerated not only flowering but also podding. Bortwhick & Parker¹⁾ stated that matured leaves are more sensible to photoperiodic stimulus than the younger ones. Reckoning from the day of treatment, (B) flowered 11 days earlier than (C). So it may also be said that a matured plant are more susceptible to photoperiodic stimulus than an im-matured one.

Table 1. Effect of the shortening of growth by short day treatment

Experimental plot	Beginning of short day treatment		Beginning of flowering					Days from flowering to podding	Diff. (A-B,C)
	Date	Days after germination	Date	Days after germination	Diff. (A-B, C)	Days after short day treatment	Diff. (C-B)		
Natural (A)			Aug. 8	77th day				21	
Late treated short day (B)	Jul. 5	43rd day	Jul. 24	62nd day	15	19		5	16
Early treated short day (C)	Jun. 1	8th day	Jul. 1	38th day	39	30	11	4	17

(2) Restraint of stem growth Dwarfishness can be seem clearly in (B) but

is extreme in (C) (Figs. 1 & 2). The similar phenomenon was already reported by many authors who mentioned that dwarfishness oc-cured as the result of the precocious differen-tiation of reproductive organ formation (Tagu-chi)⁷⁾. The short day treatment on (C) began on June 1, but for 25 days no symptom of the restraint of stem elongation was observed (Fig. 2). But the restraint was observed on (B) 5 days after the commencement of the treatment. The restraint appeared in (C) 5 days and in (B) 10 days before flowering. The short day treatment was more effective in ac-celerating flowering than commencing the restraint of stem elongation.



Fig. 1. Comparision of the apperance of soybean plants whose short day treat-ment began at various times (Photho-graphed on Sept. 20, 1953).
A) Natural day-length plot;
B) Late treated short day plot;
C) Early treated short day plot

(3) Common variation of the osmotic values according to the growth periodi-city The osmotic values of all plants belonging to (A), (B) and (C) increased gradually as the plants grew and they descended somewhat at flowering and ascend-ed again at podding. The maximum value appeared at ripening season. This com-mon tendency appeared under different climatic conditions. No peculiar seasonal

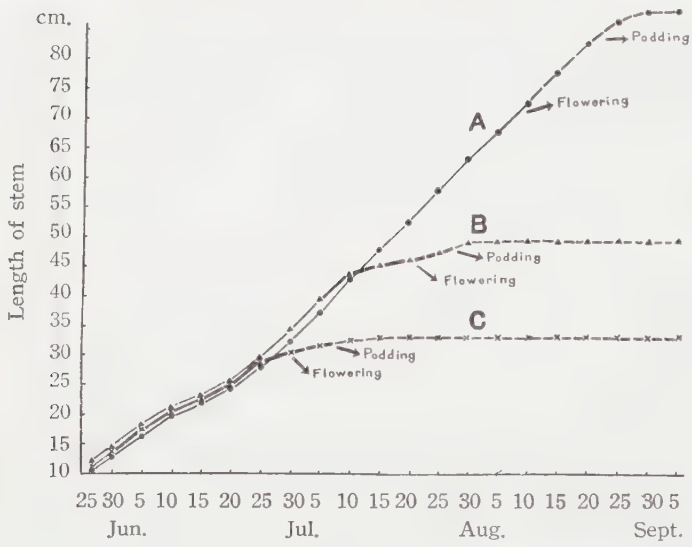


Fig. 2. Growth curve of stem of soybean plants whose short day treatment began at various times

A) Natural day-length plot; B) Late treated short day plot (commenced on Jul. 5, 1953); C) Early treated short day plot (commenced on Jun. 1, 1953)

Thick lines indicate the periods of short day treatment.

Broken lines indicate the period after flowering.

change of osmotic values was observed by the short day treatment.

Table 2. Osmotic value at the same growth period of plants whose short day treatment began at various times

Experimental plot	Flowering period			Podding period			Ripening period			Number of ripe pod
	Date	Osmotic value (KNO ₃ mol)	Diff. (A-B, C)	Date	Osmotic value (KNO ₃ mol)	Diff. (A-B, C)	Date	Osmotic value (KNO ₃ mol)	Diff. (A-B, C)	
Natural (A)	Aug. 7	0.31		Aug. 28	0.40		Sept. 22	0.45		55
Late treated short day (B)	Jul. 22	0.27	-0.04	Jul. 28	0.33	-0.07	Aug. 22	0.35	-0.10	40
Early treated short day (C)	Jul. 1	0.24	-0.07	Jul. 7	0.28	-0.12	Jul. 28	0.31	-0.14	18

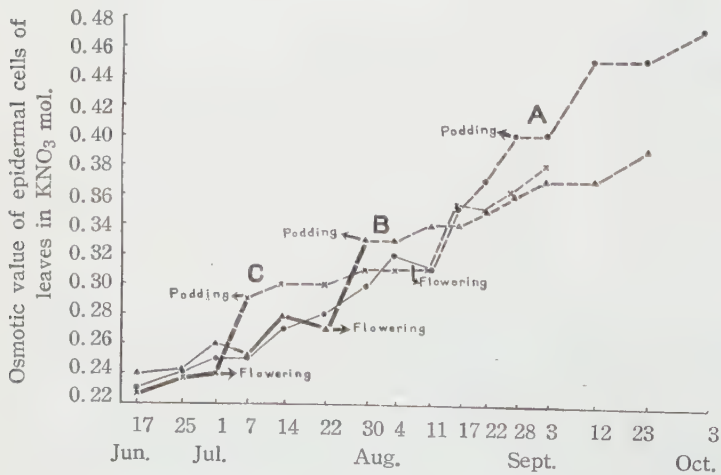


Fig. 3. Variation of the osmotic value in soybean plants whose short day treatment began at various times

A) Natural day-length plot; B) Late treated short day plot;

C) Early treated short day plot

Thick lines indicate the period of short day treatment.

Broken lines indicate the period after flowering.

(4) **Lowering effect on osmotic value at the same growth periods** Table 2 and Fig. 3 show the osmotic values of the plants of (A), (B) and (C) at the same growth periods. In Fig. 3 climate seems not to modify the growth periodical variations. The values of (C) are the lowest and those of (A) are the highest at each stages (Table 2). At each growth period (flowering, podding and ripening) the osmotic value is low if the period is attained in early age. In spite of their low osmotic value, the treated plants bore pods which contained normal seeds though the yield was less corresponding to the restraint of growth.

Discussion

The coming of flowering period is a definite physiological phenomenon wherein osmotic value temporarily lowers. At this cardinal point the osmotic value, however, differs in each plot: the value is lower in precocious plants and higher in late matured ones (Table 2). The osmotic values depend upon the length of duration of vegetative growth from germination till flowering. And the differences of the values among three plots are kept during the rest of their lives. Furthermore, these differences still increase at podding and then at ripening, as the growth stages advance. As short day treatment shortens growth periods, the treated plants become to possess lower osmotic value, in proportion to the duration of the treatment. Namely, short day treatment does not affect osmotic value which increases with the progress of vegetative growth. It can be said, from the opposite view point, that the growth periodical variation occurs independent of the comparatively low osmotic values in the treated plants. The continuance of short day treatment accelerates the differentiation of reproductive organ formation even at the diminished vegetative growth and osmotic value.

Conclusion

The effect of short day treatment accelerates the differentiation of reproductive organ formation and causes the shortening of growth periodicity. The osmotic value of a plant varies according to the length of growth duration: the normal one whose growing duration is longest has the highest value, and a heavily treated one with the shortest growth duration has the lowest. The degree of osmotic ascension caused by the aging is the function of the age of vegetative growth and is not influenced by the maturation caused by the differentiation of reproductive organs. The growth periodical variation of osmotic value regularly appears independently of both the length of growth duration and the growing season.

The writer is indebted to Mr. Yoshio Furutani of Kyushu Agricultural Experimental Station for his kindness in supplying material for this study.

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抄 録

紅藻 *Iridophycus Flaccidum* (*Irideae laminarioides*)の光合成による $C^{14}O_2$ の同化

[Beans, R. C. and Hassid, W. Z.: Assimilation of $C^{14}O_2$ by photosynthesizing Red Alga, *Iridophycus Flaccidum*., J. Biol. Chem., 212: 411~425 (1955)]

陸上植物では D-ブドウ糖と D-果糖が単糖類としては広く存在し、蔗糖、澱粉、セルロース、フラクトザンがこれらの主な寡糖類及び多糖類である。多くの海藻では陸上植物の有している様な炭水化物は全然有していないか、有していても比較的少量で、マンニトール、フロリドサイド（ガラクトースのグリセリン・エステル）グリセリンマンノサイド、及びマンノウロン酸、L-フコース、D-ガラクトース残基から成る多糖類が主な炭水化物成分である。かように、陸上植物と海藻では、それらの主な炭水化物成分が異つているので、光合成に於いても、炭素が炭水化物を生成する過程が異つているか否かを、紅藻 *Iridophycus flaccidum* で検してみた。

材料は California の Moss Beach の tidal rocks で採集した果胞子のできてない生長期にある葉状体（同地で採集した同材料については、主な炭水化物成分である粘物質即ちガラクトサンの硫酸エステル及びフロリドサイドの構造等）に関し、1933年以來 Hassid の多くの報告がある。）を 6~24 cm³, 0.25~1.0 gm の切片にしたものを用い。

それを $C^{14}O_2$ を導き入れられる Chamber を有する光合成装置中に置いて実験を行つた。

光合成の最初の 8 秒間にグリセリン酸磷酸セドヘプチュロース磷酸、果糖一磷酸、ブドウ糖一磷酸が、8~15 秒の間に、 α -グリセリン磷酸ウリジン=磷酸ブドウ糖が、15~30 秒間にウリジン=磷酸ガラクトース、フロリドサイドが label され、その他フロリドサイド磷酸、グリシン、セリン、アラニン、グリセリン酸、グルコン酸、リンゴ酸、グルタミン酸、アスパラギン酸も label された。 C^{14} の蓄積の最も多いものはフロリドサイドであつた。これのガラクトースとグリセリンの C^{14} 量の比は 4 回の実験においてすべて、略 2 に近い値を示した。*I. flaccidum* の光合成の初期の段階の生成物は、クロレラや高等植物 (Benson et al and Calvin 1950~1952) の場合と同じであり、それらの形成の速度も同様であつた。又、フロリドサイドは高等植物の蔗糖に相当し、この紅藻の主な貯蔵炭水化物であると思われる。

(野村一郎)

藓類数種の染色体

VIII ツヤゴケ属及び他の2属の染色体と性分化*

矢野孝二**

Koji YANO: On the Chromosomes in Some Mosses.

VIII Chromosomes and Sex-Differentiation of *Entodon* and Other Two Genera

1955 年 1 月 12 日受付

ツヤゴケ (*Entoden*), イワダレゴケ (*Hylocomium*), ヒツジゴケ (*Brachythecium*) 属には同一属内に雌雄同株及び異株の種があるので, その性分化と染色体構成との関係を明らかにすることは興味がある。それで筆者は既にこれ等3属の若

干種の染色体について報告しているが(矢野1952, '53), 更に今回新たに数種の染色体観察を加へこれ等3属に於ける染色体と性分化との関係を比較検討した。その結果これ等の関係を多少明らかにすることが出来たので以下報告する。

Table I

Species examined	Sex	Chr. nos. (n)	Karyotypes	Localities
<i>Brachythecium populeum</i> (Hedw.) Br. eur.	♂	10	V(H)+3V+5(4v+j)+m(h)	燕温泉, 青海黒姫山, 越前: 金沢市
<i>B. decurrentifolium</i> * Broth.	♀, ♂	10	V(H)+2V+6(4v+2j)+m(h)	青海黒姫山
<i>B. Buchanani</i> (Hook.) Jaeg. var. <i>japonicum</i> Card.*	♂	10	V(H)+2V+6(4v+2j)+m(h)	高田市
<i>Entodon Challengeri</i> Par.	♂	11	V(H)+V+8(6v+2j)+m(h)	下野: 中川村 土佐: 室戸町
<i>E. chloroticus</i> Besch.*	♂	11	V(H)+V+8(6v+2j)+m(h)	燕温泉, 高田市
<i>E. ramulosus</i> Mitt.	♀, ♂	11	V(H)+V+8(6v+2j)+m(h)	信濃: 五地藏岳 越前: 金沢市
<i>Hylocomium cavifolium</i> Broth.	♀, ♂	6	V(H)+2V+2J+m(h)	谷浜村
<i>H. proliferum</i> (L.) Lindb.*	Sterile	12	2V(H)+4V+4J+m+m(h)	妙高山, 苗場山, 青海黒姫山

註. 国名を省略したものはすべて越後 * 第II, IV 報にて一部を報告

* 文科研論文

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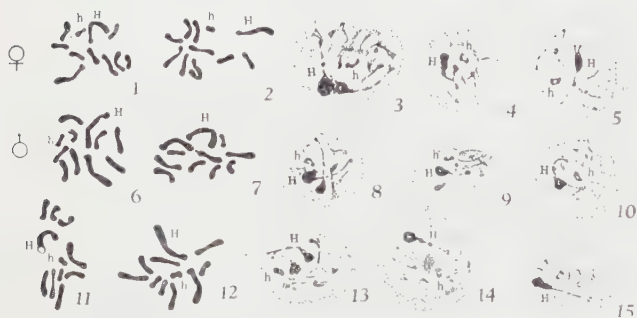
研究に用いた藓の種名並びに採集地は Table I に示す。固定並びに染色法は前報告(矢野 1952)の場合と同様である。

観 察

1) *ENTODON* 属

雌雄異株の *E. ramulosus*, 雌雄同株の *E. Challengeri*, *E. chloroticus* の 3 種について観察を行った。尤もこれ等 3 種のうち最後の *E. chloroticus* の染色体については既に筆者 (1952) がその染色体数を $n=11$ と算定しているが、今回他との比較の為再観察を行ったものである。

雌雄異株の *E. ramulosus* では雌株及び雄株の間に染色体の形態、異常凝縮の状態に差異が認められず、その核型は雌雄共に $K(n)=11=V(H)+V+8(6v+2j)+m(h)$ で示される。即ち本種には雌雄の間で形態を異にする性染色体は認められない (Figs. 1-10)。又雌雄共に最大の染色体はその中央に一次狭窄を有しその形は V 字形であり、亦その一腕の尾部に二次狭窄を有する。そしてこれは異質染色体 H であり、その異常凝縮部は：



Figs. 1-15. *Entodon* 属 3 種の配偶体の染色体と異常凝縮 (Chromosomes and heteropycnosis in gametophytes of genus *Entodon* examined)

1-5. *E. ramulosus* ♀ 6-10. Ditto ♂
11-15. *E. Challengeri* ♂ × 1330

次狭窄をもたない方の腕の大部分と二次狭窄を有する方の腕の尾部であつて、これ等の部分に由来する顕著な染色質塊は休止核に於いても明瞭に認められる。最小の染色体 (m) は他の蘚の場合と同様に異質染色体で h で示される。この h の異常凝縮は本種では殊に明瞭であつて、これは休止核では常に仁の中心部に位置し、且殆んど中期にに於けると同様な形態を保っている。即ちこの h は Nukleolinus を作るので、いわゆる “Nukleolinus-Chromosomen” である。*

こゝに興味あることは 9 個の常染色体のうち最大の 1 染色体が幾分 H に類似した性状を示すことである。即ちこの染色体の大きさはほぼ H に近く、その形も V 字形で、尚一腕の尾部にしばしば二次狭窄が認められ、従つてその形態は H に近似している。その上この染色体は前期の核中で他の常染色体よりも早期に形態を整え、H と共に仁に接して現われる。然し前期核中における染色度は H に比すれば弱く、また休止核では染色質塊を残さない。従つてこの染色体は H とは異なるのでこゝでは一応常染色体として示すことにした。其他の 8 個の常染色体は何れも小形である為それ等の狭窄の位置は充分詳かになし得ないが、およそ 6 個の v 及び 2 個の j より成つている。

雌雄同株の 2 種 *E. Challengeri*, *E. chloroticus* は前記の *E. ramulosus* とは雌雄性の分化を異にするにもかかわらず、筆者の観察した範囲では染色体の形態及び異常凝縮の状態は Figs. 11-15 に示す如く、殆んど全く前記種のそれ等と同様であつて根本的な差異は認め難い。

2) *BRACHYTHECIUM* 属

筆者は先に (矢野 1954 b) 本属の雌雄異株の 2 種 *B. decurrentifolium*, *B. Buchanani* var. *japonicum* が $n=10$ であることを報告した。今回新たに観察を行った *B. populeum* は同じく $n=10$ であるがこれは明瞭な雌雄同株である。そこでこの様に性分化を異にする両者の染色体の相違を明らかにする為既報の雌雄異株のものを再観察し、雌雄同株の本種と比較した。

その結果両者の間に核型その他について多少の差異を認めることが出来た。即ち雌雄異株の 2 種の核型は何れも ♀, ♂ $K(n)=10=V(H)+2V+6(4v+2j)+m(h)$, 雌雄同株の本種の核型は ♂ $K(n)=10=V(H)+3V+5(4v+j)+m(h)$ である。即ちその相違点は前者 (Figs. 16, 17) では

* 辰野 (1954a, b) はケゼニゴケ (*Dumortiera hirsuta*) の m 染色体が休止核に於て仁の中に入つて Nukleolinus を作ることを認め、この m を Nukleolinus-Chromosomen と名づけた。



Figs. 16-28. *Brachythecium populeum* 外 2 種の染色体, 異常凝縮及び減数分裂

(Chromosomes, heteropycnosis and meiosis in *Brachythecium populeum* and other two species)

16-20. Metaphase chromosomes in gametophytes.

16. *Brachythecium decurrentifolium*. ♀. 17. *B. Buchanani* var. *japonicum*. ♂. 18-20. *B. populeum*. ♀.

21-25. Heteropycnosis in prophase (21, 22, 23) or resting (24, 25) nuclei of gametophytes of *B. populeum*.

26-28. Meiotic chromosomes at 1st metaphase (26) and anaphase (27, 28) in SMC's of *B. populeum*. × 1330



Figs. 29-44. *Hylocomium* 属の配偶体の染色体と異常凝縮

(Chromosomes and heteropycnosis in gametophytes of genus *Hylocomium* examined)

29-33. *H. cavifolium* ♀. 34-38. Ditto ♂.

39-44. *H. proliferum*. 44. A nuclear plate showing $n=6$ which was found among the normal cells with $n=12$ of *H. proliferum*. × 1330

常染色体のうち大形の V が 2 個, 後者 (Figs. 18-20) では 1 個多く 3 個である。そのかわり後者では小形の j 染色体が 1 個少い。

本種の異質染色体は H, h の音 1 個で, これ等の形態及び異常凝縮性は既報雌雄異株の 2 種と何等異るところが認められない (Figs. 21-25)。更に既報種では常染色体中最大の V が幾分異質染色体的傾向を示すことを報告したが, 本種でも 3 個の大形の V 形常染色体中の特に大きい 1 個が異質染色体的傾向を示す。この染色体は一腕の尾部に明瞭な二次狭窄を有し, その形態は H と殆んど全く同様である。然し H に比すればその凝縮性は微弱であつて休止核では殆んど認められなくなるので, こゝでは一応常染色体として示した。即ち本属においても前記の *Entodon* 属に見られたと同様な特異な異常凝縮性を示す 1 染色体が見られるわけである。

尙本種の子嚢体における減数第一分裂中期には 10 個の等対の Bivalents があり, 不等対は認められない。後期には何れも同大の染色体に分離して両極に向う (Figs. 26-28)。

3) HYLOCOMIUM 属

先に筆者は *H. proliferum* の染色体数 $n=12$ を報告したが (矢野 1952), 今回同属の *H. cavifolium* の観察を行い, それが $n=6$ であり, 本属に倍数関係があることがわかつたので, こゝに更めて両種の染色体の比較観察を行つた。

H. cavifolium ($n=6$) は明瞭に雌雄異株である。中期染色体の形態及び異質染色体 H, h の前期における異常凝縮の状態は雌雄の間で何等の差異が認められず (Figs. 29-38)

従つて本種には性染色体は認められない。即ち本種の核型は雌雄共に $K(n)=6=V(H)+2V+2J+m(h)$ で示される。そのうち H は核板中最大、 V 字形の染色体で、既報の他の蘚の H と同様に一腕の尾部に二次狭窄を有する。且この H の異常凝縮部は二次狭窄を有しない方の腕の大部分及びこれを有する方の腕の尾部である。又 h は微小な染色体 m であつて、その全部が異常凝縮を示し、且これは休止核では仁の中心部に Nukleolus をつくるので Nukleolus-Chromosomen である。其他の常染色体は 2 個の V 及び 2 個の J より成る。

H. proliferum は雌雄異株とされているが本研究に用いられた材料は生殖器をつけず、その性別は不詳であつた。本種の染色体数は前報告 (矢野 1952) の如く $n=12$ で前種 *H. cavifolium* ($n=6$) に比すればその二倍種である (Figs. 39-44)。12 個の染色体中 2 個は他より際立つて大きな V 形の異質染色体 H である。ところが注意すべきことはこれ等 2 個の H の形態及び異常凝縮性には互に明瞭な差異があることである。即ち 1 個は前記一倍種の H と殆んど同じ形態と異常凝縮性を示すが、他方はこれに比して幾分小さく、且時に二次狭窄が不明瞭な場合もあり、亦異常凝縮も弱く休止核における染色塊も小さい。然し両 H の異常凝縮を示す部分は何れも二次狭窄をもつ腕の端部及びこれをもたない方の腕の大部分であつて、両 H の間に根本的な差異は認め難い。亦本種には 2 個の m があるが、確実に異常凝縮を示すのはそのうちの 1 個で他はこれを示すか否か未だ詳かでない。其他の常染色体は 4 個の V 及び 4 個の J より成る。従つて本種の核型は $K(n)=12=2V(H)+4V+4J+m+m(h)$ で示される。即ち本種は前記の一倍種とは異なる同形の染色体を二重にもっている。

尙 Fig. 44 は本種の配偶体で $n=12$ の組織の間に偶然に見出された $n=6$ を示す核板である。この様な異数を示す核板は本種の観察中時々見られたが、この核板の染色体はその大きさ形態等が殆んど全く一倍種 *cavifolium* のそれ等に似ていることは興味がある。この様な細胞が見られることは本種が *cavifolium* の如き染色体組をもつたものから導かれた二倍種であることを示すものではあるまいか。

考 察

蘚類における天然の倍數性とその雌雄性の分化との関係については種々場合が見られる。そのうち一倍体が雌雄異株で二倍体が雌雄同株となつている場合が最も多く、筆者も *Heterophyllum* (1951), *Pholia*, *Thamnium* (1953 a), *Polytrichum* (1953 b), *Brachythecium* (1954 a) の諸属でこれを報告している。これ等の諸属では雌雄同株の二倍体は一倍体の雌株及び雄株の染色体を完全に併有して、例へば一倍体の雌雄の間でその H に形態的差異がある場合には (これ等は性染色体 X, Y である) 二倍体の 2 個の H の間にもこれと同様な差異が見られ (*Polytrichum*)、又一倍体の雌雄の H に形態的相違がない場合には二倍体の 2 個の H もこれに対応して互に同形である (*Thamnium*, *Brachythecium*)。それでこれ等の二倍体は一倍体から Apospory 又は減数分裂の異常による二分胞子形成によつて導かれた倍數体に起原するものと考えられる (矢野 1951, '53 a, '53 b, '54 a)。

ところが今回観察された *Hylocomium* 属の倍數性の性分化はこれと異り *H. proliferum* ($n=12$) は *H. cavifolium* ($n=6$) の正二倍体であるにもかかわらず両者は共に雌雄異株である。そして一倍体 *cavifolium* の雌株及び雄株の H の間には形態的相違が認められないにもかかわらず二倍体 *proliferum* の 2 個の H 間には形態、殊に異常凝縮性に明らかな差異が見られる。かくの如く二倍体において相対応すべき異質染色体間に異常凝縮性の分化が見られる例は既に二三の若種で知られているが、その成因について二様の説明がなされている。即ちその一つは *Pellia borealis* (Lorbeer 1934, Jachimsky 1935) の H の場合で、それは異つた異質染色体を有する二種間の雑種に由来するものとして、他はケゼニゴケ (*Dumortiera hirsuta*) の h の場合で、これでは天然に創生された同質倍數体が長年月の間に安定性を得る課程において h 間に分化が生じたものとされている (辰野 1938, '41, '52)。筆者が今回研究したイワダレゴケ (*H. proliferum*) の 2 個の H 間の分化については、本種の核型が同質二倍体に近いこと等から考察して、おそらくケゼニゴケの場合と同様な課程の結果と見ることが妥当であらう。

う。即ち本種の 2 個の H は本来相同なものであつたが、種の安定性獲得の課程において両者間に分化を生じたものであろう。次に本種が一般の正二倍体の場合と異り雌雄異株となつてゐることは更に解釈の困難な問題であるが、これは或は長年月にわたる H 間の分化の過程において、あるものは雌性として他のものは雄性染色体として分化しているのではあるまいか、これ等の点については更に今後の研究によつて明かにしたいと思ふ。

今回研究された *Brachythecium*, *Entodon* 両属には Table I に示す如く、同一属内に染色体数及び異質染色体が殆んど全く等しいにもかかわらず雌雄同株又は異株を示す種がある。このような例は苔類に於ても屢々報告されているが、その性

分化の機構は未だ明確にされていない(辰野 1941)。筆者の観察した上記両属の各種ではその染色体組のうちにそれぞれ H の他に形態的に H に似てやゝ異常凝縮性において H と異なる特異な染色体が 1 個づゝ見られる。それで或はこの特異な染色体が H と共に性分化に関与し、その為に或は雌雄同株となる場合も生ずるのではあるまいか、これは今後の研究によつて明らかにしたいところである。

本研究の遂行に當つて御指導をいただいた辰野博士、並びに研究材料の採集につき協力をいただいた牛木博氏に対し謝意を表します。

Résumé

1) The karyotype analysis of mosses, eight species belonging to three genera, was carried out:

<i>Entodon Challengeri</i>	♀	$K(n)=11=V(H)+V+8(6v+2j)+m(h)$
<i>E. chloroticus</i> *	♀	$K(n)=11=V(H)+V+8(6v+2j)+m(h)$
<i>E. ramulosus</i>	♀, ♂	$K(n)=11=V(H)+V+8(6v+2j)+m(h)$
<i>Brachythecium populeum</i>	♀	$K(n)=10=V(H)+3V+5(4v+j)+m(h)$
<i>B. decurrentifolium</i> *	♀, ♂	$K(n)=10=V(H)+2V+6(4v+2j)+m(h)$
<i>B. Buchanani</i> var. <i>japonicum</i> *	♂	$K(n)=10=V(H)+2V+6(4v+2j)+m(h)$
<i>Hylocomium cavifolium</i>	♀, ♂	$K(n)=6=V(H)+2V+2J+m(h)$
<i>H. proliferum</i> *	(sterile)	$K(n)=12=2V(H)+4V+2J+m+m(h)$

2) By three dioecious species, i.e. *Entodon ramulosus*, *Brachythecium decurrentifolium*, *Hylocomium cavifolium*, the chromosomes and the heteropycnosis in prophase nuclei of both male and female gametophytes were carefully compared with one another, but no sex chromosomes differed in morphology have been found.

3) The chromosome numbers in the genus *Hylocomium* studied in this paper show a polyploid series of $n=6, 12$. The diploid species *H. proliferum* has two sets of chromosome complement which is approximately similar in the morphology of chromosomes to that of the monoploid species *H. cavifolium*.

* These species were already reported in part in the series II and VI (Yano 1952, 1954b) of the present paper.

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Triticum Timopheevi × *Secale africanum* F₂ 植物の細胞遺伝学的研究*

中 島 吾 一**

Goichi NAKAJIMA: Cytogenetical Studies on the F₂ Plants of *Triticum Timopheevi* × *Secale africanum*.

1955 年 1 月 21 受付日

緒 言

1952 年に得た *Triticum Timopheevi* × *Secale africanum* F₁ 植物 (*TimoSaF*₁) 69 個体は殆ど完全なる不稔であつたが、その 395 穂、約 129000

差異があるためである。而して F₂ 植物は稈長においては両者ともに F₁ に劣り、穂長および芒長においては、No. 1 の個体は F₁ に優り、No. 2 の個体においては劣つて居た。小穂の密度においては No. 2 の個体は F₁ に優り、No. 1 の個体

Table 1 External characters of F₂ compared to the parental plants, *TimoSaF*₁

Characters Plants	Number of chromo- somes (2n)	Length of culms (cm)	Length of spikes (cm)	Length of awns (cm)	Number of spikelets per spike	Spike den- sity	Number of flowers per spikelets	Number of tillers
<i>TimoSaF</i> ₁	21	125.04	10.31	3.85	32.65	3.18	3	145.89
<i>TimoSaF</i> ₂ -1	39	88.95	12.60	6.30	25.10	1.99	3	72.00
" -2	25	68.06	6.18	2.04	22.00	3.56	2	79.00

の小穂から 4 粒の種子を得た (Nakajima 1955)。この 4 粒の中 2 粒は貯蔵中に虫害を受け、残りの 2 粒を 1953 年 10 月に播種して 2 本の F₂ 植物 (*TimoSaF*₂) を得た。ここにこれらの F₂ 植物について行つた細胞遺伝学的研究の結果を述べる。

研究の方法は筆者が今迄に報じた小麦ライ属間雑種の細胞遺伝学的研究と同様である。図の原倍率は体細胞染色体に対しては×1000、花粉母細胞の染色体に対しては×770 である。

観察の結果および考察

F₂ 植物の外部形質: F₂ 植物 2 個体の外部諸形質を親植物 (F₁) のそれと比較すれば第 1 表のようである。

第 1 表に示すように F₂ 植物 2 個体の間には各形質についてかなりの差異を示した。これは両者間に染色体数、従つて染色体構成において著しい

Photo. 1. Spikes of *T. Timopheevi* × *S. africanum* F₂ (*TimoSaF*₂) plants, left, No. 1 (2n=39), right, No. 2 (2n=25). × $\frac{1}{3}$

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は劣つて居た。1 穂の小穂数および分蘖数は何れも F_2 が F_1 に劣つて居た。概括的に見れば、No. 2 の個体は小型であるだけでやゝ F_1 に似て居るが、No. 1 の個体は著しく F_1 と異なつて居た (Photo. 1)。

TimoSaF₂-1 植物の花粉母細胞における成熟分裂：この個体の根端細胞において $2n=39$ の染色

るならば、この *TimoSaF₂-1* 植物の第 1 成熟分裂中期において、少なくとも最高 16II が観察されるであろうが、小麦ライの雑種においては、2 価染色体の中にはしばしば早期に分裂するものもあるから、14II が最高数として観察されたものであろう。

Table 2 Frequencies of bivalents and univalents at heterotypic metaphase in meiosis of the PMC's of *TimoSaF₂-1* ($2n=39$) plant

Individuals	Bivalents and univalents										Mode	Total
	14II + 11I	13II + 13I	12II + 15I	11II + 17I	10II + 19I	9II + 21I	8II + 23I	7II + 25I	6II + 27I	5II + 29I		
<i>TimoSaF₂-1</i>	1	6	9	31	67	159	113	71	21	1	9II	479
%	0.21	1.25	1.88	6.47	13.99	33.19	23.59	14.82	4.83	0.21	33.19	99.99

体が観察された (Fig. 1)。その花粉母細胞においてもまた $2n$ 数として 39 の染色体を観察した (Figs. 2~5)。而して第 1 成熟分裂中期において 5~14 の 2 価染色体と、11~29 の 1 価染色体とを観察した。1 花粉母細胞中に見出される 2 価染色体数の頻度は第 2 表の通りである。

第 2 表によつて明かなように 1 花粉母細胞中に見出される 2 価染色体数は 9 の場合が頂数を示した。2 価染色体は何れも等大の 2 染色体からなる緊密な結合をなす環状のもの、端部において結合する棒状のものが見られた。而して 1 花粉母細胞中に見出される数は概して後者の方が前者よりも多かつた。2 価染色体の外にごく稀に 3 価染色体も観察されたが、4 価染色体は観察されなかつた。而して 3 価染色体の形態は V 字形であつた。

TimoSaF₂-1 個体の染色体数 $2n=39$ は、親植物の F_1 に生じた個配子の中には同数、即ち 19 と 20 の染色体を持つものの結合によつてもたらされたものであろう (*TimoSaF₁* の PMC の第 2 成熟分裂においては観察数 (353) の少なかつたためでもあろうが、19 以上の染色体を持つ核板は観察されて居らない (Nakajima³⁾)。この染色体数 19 および 20 は F_1 の染色体数 $2n=21$ より 1~2 個少ない数であつて、*T. turgidum* × *S. cereale* F_1 (Nakajima^{1), 2)} におけると同様に受精力を持つて居ると考えられる。もし上の想定通りであ

この植物の染色体数 $2n=39$ のゲノム式はかりに *S. africanum* のゲノムを R^a とすれば、AA GGR^aR^a-3 となり欠けて居る 3 染色体は 3 組のゲノムの中、何れか 1 組のゲノムのみに属するものであるならば (*T. turgidum* × *S. cereale* F_1 においては 3 組のゲノムの中 2 組が完全であれば、残りの 1 組のゲノムは 3 染色体を欠いて居ても生活力並びに受精能力を持つて居た (Nakajima²⁾)。この植物の外部形態は AAGG, AAR^aR^a あるいは GGR^aR^a の外に、残りの 11 個の染色体の加わつたものに由来するものであらねばならない。然るにこの植物の外部形態は少くとも AAGG ゲノムを完全に持つもの、即ち *T. Timopheevi* の形質を強く表はしたものとと思われぬ (photo. 1)。さればこの欠除した 3 個の染色体は少なくとも 2 組以上のゲノムに属するもの (G および Ra?) であらう。而してこの欠除した染色体の種々なる組合せの一つとして、この *TimoSaF₂-1* 植物が生じたものであると考えられる。

TimoSaF₂-2 植物の花粉母細胞における成熟分裂：この個体の根端細胞において $2n=25$ の染色体が観察された (Fig. 6)。その花粉母細胞においてもまた $2n$ 数として 25 の染色体を観察した (Figs. 7~13)。而して第 1 成熟分裂中期において、3~9 の 2 価染色体と 7~19 の 1 価染色体とを観察した。而して 1 花粉母細胞中に見出される 2 価染色体数の頻度は第 3 表の通りである。

第3表によつて明かなように、1花粉母細胞中に見出される2価染色体の数は6の場合が最も多い。2価染色体は何れも等大の2染色体からなり、環状のものと棒状のものとが見られた。而し

ものと考えられよう。

この *TimoSaF₂-2* 植物の第1成熟分裂における最高9の2価染色体は *AGRa* ゲノムの中、何れのゲノムに属する染色体の結合によつて生じた

Table 3. Frequencies of bivalents and univalents at heterotypic metaphase in meiosis of the PMC's of *TimoSaF₂-2* ($2n=25$) plant

Individuals	Bivalents and univalents							Mode	Total
	9 _{II} + 7 _I	8 _{II} + 9 _I	7 _{II} + 11 _I	6 _{II} + 13 _I	5 _{II} + 15 _I	4 _{II} + 17 _I	3 _{II} + 19 _I		
<i>TimoSaF₂-2</i>	13	70	102	153	86	38	24	6 _{II}	486
%	2.67	14.40	20.99	31.48	17.70	7.80	4.94	31.48	99.98

て1花粉母細胞中に見出される数は前者の方が後者よりも多かつた。3価および4価染色体は観察されなかつた。

TimoSaF₂-2 個体の染色体数 $2n=25$ は、親植物の F_1 に生じたほぼ同数、即ち12と13或は11と14の染色体を持つた配偶子の結合によつてもたらされたものと考えるべきであろう。これらの配偶子のできる割合を F_1 の花粉母細胞の成熟分裂について見れば、12および13の染色体を持つものは18.41および11.61計30.02%、また11および14の染色体を持つものは54.68および5.67計60.35%である(Nakajima³⁾)。胚嚢母細胞の成熟分裂においてもまたこれと同様であるとすれば12と13の染色体を持つ配偶子の結合確率(0.2137)は、11と14の結合確率(0.3100)よりも小さい。即ちこの *TimoSaF₂-2* 個体は11と14の染色体を持つ配偶子の結合によつて生し

たものであるかを明かにすることは困難である。さればこの植物のゲノム構成を明かにすることは出来なかつた。

第2成熟分裂においても、第1分裂におけると同様に多少不規則性を示し後期において laggards も見られた。

四分子は4細胞からなる場合が最も多く、その形態も正常のものが最も多い。また四分子を形成する各細胞には不規則分裂に由来する小核を有する場合が多く、その数は0~7で4~5が頂数を示した。

これら *TimoSaF₂-1* および2の個体では成熟分裂の不規則の結果生ずる花柄は染色体構成および大きさを異にするもの多く、ために受精力を欠くものであり、従つて葯も裂開するに至らず完全なる不稔性を示した。

Resume

1. In the present report, the results of cytogenetical studies of 2 F_2 plants (*TimoSaF₂*) of *T. Timopheevi* × *S. africanum* F_1 were described.

2. The number of somatic chromosomes of the 2 individuals was found to be 39 (*TimoSaF₂-1*) and 25 (*TimoSaF₂-2*) respectively.

3. External characters of the *TimoSaF₂* plant were quite different from those of F_1 as shown in the Table 1 and Photo. 1.

4. The number of bivalents in one PMC at heterotypic metaphase varied 5~14 in the *TimoSaF₂-1* and 3~9 in the *TimoSaF₂-2*. The frequency of bivalents in the PMC's of the F_2 plants was tabulated in the Tables 2 and 3; occurrence of 9_{II} (*TimoSaF₂-1*) and 6_{II} (*TimoSaF₂-2*) appeared to be the mode respectively.

5. The 2 individuals of *TimoSaF₂* plant were completely sterile.

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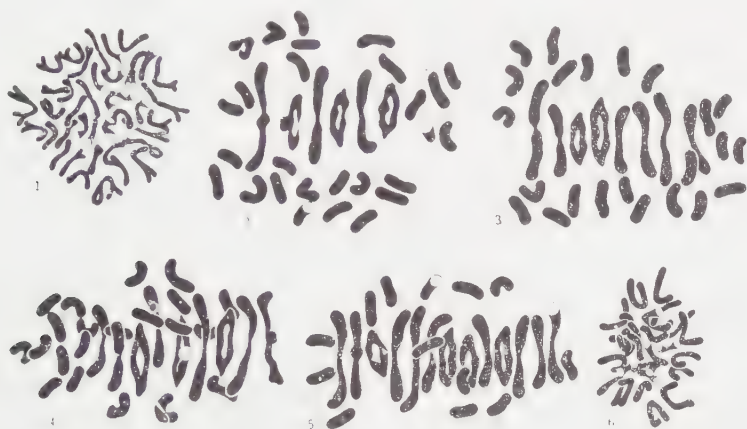


Fig. 1. Somatic chromosomes in root tip cell of *TimoSaF₂-1*, $2n=39$. $\times 1000$

Figs. 2~5. Meiosis in PMC's of *TimoSaF₂-1*. Heterotypic division. $\times 770$

2. Metaphase, side view, drawn separately, $6\text{II}+27\text{I}$; 3. do. $8\text{II}+23\text{I}$; 4. do. $9\text{II}+31\text{I}$; 5. do. $13\text{II}+13\text{I}$.



Fig. 6. Somatic chromosomes in root tip cell of *TimoSaF₂-2*, $3n=25$. $\times 1000$

Figs. 7~14. Meiosis in PMC's of *TimoSaF₂-2*. Heterotypic division. $\times 770$

7. Metaphase, side view, $3\text{II}+19\text{I}$; 8. do. $4\text{II}+17\text{I}$; 9. do. $5\text{II}+15\text{I}$; 10. do. $6\text{II}+13\text{I}$; 11. do. $7\text{II}+11\text{I}$; 12. do. $8\text{II}+9\text{I}$; 13. do. $9\text{II}+7\text{I}$; 14. Ana-telophase, side view, 5 laggards.

キイチゴ属雑種の研究 I

カデイチゴ♀ × クサイチゴ♂について

神 野 太 郎*

Taro JINNO: Studies on the Hybrid in *Rubus* I. *R. trifidus* Thunb.

♀ × *R. hirsutus* Thunb. ♂

1955 年 3 月 10 日受付

緒 言

キイチゴ属植物の雑種に関する研究は Wellington (1913), Thomas (1940), Crane (1940), Vararama (1951) 等によりてなされており, Thomas は数種のものについてゲノム分析を行つている。これらの材料はみな外国産のものであり, 本邦産のキイチゴ属植物の雑種については未だ研究がなされていない。筆者は 1951 年以来キイチゴ属植物で種間交配を行ひ数種の雑種を得たが, 今回はこれら雑種のうち一雑種について報告する。

材料及び方法

雑種の両親即ち *R. trifidus* Thunb. (カデイチゴ) 及び *R. hirsutus* Thunb. (クサイチゴ) はどちらも 2 倍体で染色体数は $2n=14$ (Jinno 1951) で, 前者は松山市附近で栽培されていたものである。この雑種は *R. trifidus* を母本とし *R. hirsutus* を父本として作つた雑種で, 種子の稔性は良好である。1951 年春交配して得た種子を約 1 ヶ月後角鉢に播種し, これが 1, 2 寸伸びた頃露地にうつして栽培した。この雑種は 1953 年早春開花した。この雑種における花粉母細胞の減数分裂の観察には, 若い蕾をナワシン液で固定し, パフフィン切片法で切片をつくり, ハイデンハイン氏鉄剛紫・ヘマトキシンリンで染色したものを用いた。この F_1 雑種と比較するため両親植物は雑種と同じく, よく耕作された畑地に栽培した。

観 察

生育: 筆者はキイチゴ属数種の種間交配により数種類の雑種の種子を得たが, 種子の発芽はこの雑種のものが最も良好であつた。又発芽後の生育もこの雑種が他の雑種に比して最も旺盛で, 播種してより約 5 ヶ月後には草丈が 25 cm に生育し, 根茎をひいて盛に無性繁殖をする。播種してより 1 年後には無性芽の覆う地面は約 0.5 m² に及び, 更に 1 年後即ち 1953 年 9 月の調査では無性芽のおおる地面は元株より南方 3.2 m, 西北方 3 m で推定被覆面積 28 m², 根茎の不定芽より生じた株数約 200 に及び, よく生育した株の草丈は 1.80 m に達している。この雑種の両親植物である *R. trifidus* 及び *R. hirsutus* も根茎をひいて無性繁殖を行うが, この雑種はこれら両親植物よりも無性繁殖力が旺盛である。

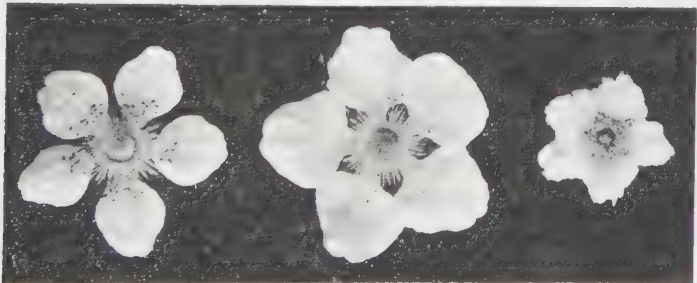
形態: この F_1 のしめす種々の形質について両親植物と比較して表示したのが第 1 表である。この表でみると雑種の形質が両親の形質の中間を示すもの, 両親中何れか一方の形質に似るもの, 及び両親のいづれよりも強くその性質をあらわすものがある。これらのうち第 1 表でみる如く, 両親の中間の形質を現す場合が最も多い。雑種の器官が両親のいづれよりも大きくなるものに次のものがある。1) 花の大きさ: 両親植物である *R. trifidus* 及び *R. hirsutus* の花の直径は, 前者が約 3.4 cm, 後者が約 4.8 cm であるが雑種の花の直径は両親植物のいづれよりも大きく約 5.8 cm をしめす (第 1 図)。2) 花弁の基部角度: *R. hirsutus* の花弁は大体楕円形をなし花弁の基部角度が約 103° であり, *R. trifidus* では花弁の形

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第 1 表 *R. trifidus*, *R. hirsutus* 及び雑種の形態比較

器官	項目	植 物	<i>R. trifidus</i>	<i>R. hirsutus</i>	hybrid
花	直 径		3.4 cm	4.8 cm	5.7 cm
	花 序		聚 散 花 序	単 頂 花 序	聚 散 花 序
	花 弁 の 皺		あ り	な し	な し
	花弁の基部角度		128°	103°	137°
	花弁の欠刻		周縁に僅かあり	な し	先端に僅かあり
	花糸の長さ(長いもの)		4~5 mm	9~10 mm	6~7 mm
葉	萼 の 展 開		平 開	反 卷	平 開
	葉 身 の 形		7 裂掌状単葉	3又は5羽状複葉	3~5 掌状複葉
	小 葉 片 の 形			長 楕 円 形	卵 形
	鋸 歯		重 鋸 歯	重 鋸 歯	重 鋸 歯
	綿 毛		な し	あ り	あ り
	棘 刺		な し	あ り	なし(稀にある)
茎	托 葉		長楕円形, 欠刻あり	披針形, 全縁	中 間 型
	丈 高		180 cm 内外	50 cm 内外	180 cm 内外
	直 径 (比較的大なるもの)		1.8 cm	0.5 cm	1.8 cm
	腺毛の分布(1 cm ²)		僅 少	155	89
	棘 刺		な し	あ り	なし(稀にあり)
花 毛	形		先 端 膨 大	先 端 尖 る	中 間 型
	長さ(長大なるもの)		0.41 mm	0.87 mm	0.65 mm

第 1 図 両親及び雑種の花の比較



左; *R. hirsutus* 中央; hybrid 右; *R. trifidus*

がほぼ円形で基部角度が約 128° であるが、この雑種の花弁基部角度は更に大きくなって約 137° である。

雑種の形態が両親の何れか一方のみの形態をあらわすものとして、*R. trifidus* に似るものには、

1) 花序: *R. hirsutus* は単頂花, *R. trifidus* は聚散花序であるが、この雑種は *R. trifidus* のごとき聚散花序をなす。2) 萼の展開: 花が充分開いてくると *R. hirsutus* は萼が反巻してくるが *R. trifidus* は一平面に展開している。この雑種の萼

は *R. trifidus* のごとく一平面に展開している。
 3) 茎の高さ及び太さ: *R. hirsutus* は草木状でよく生育したもので高さ約 80cm, 茎の直径約 0.4 cm 内外であり, *R. trifidus* は高さ約 180 cm, 直径約 1.8 cm 内外の灌木状だが, この雑種は *R. trifidus* の如く高さ約 180 cm, 直径約 1.8 cm 内外の灌木状となる。4) 棘刺: *R. hirsutus* は茎及び葉柄に棘刺があるが, *R. trifidus* は棘刺を全く生じない。この雑種は茎及び葉柄に稀に棘刺を生じる程度でほとんどこれを生じない。次に *R. hirsutus* の形態をしめすものとして、1) 花卉の皺: *R. hirsutus* は花卉の表面が平滑であり, *R. trifidus* は花卉に皺があるため表面がちぢみの様になっているが, この雑種は *R. hirsutus* の如く平滑である。2) 葉の綿毛: 充分生育した葉において *R. hirsutus* は綿毛を有し, *R. trifidus* にはこれがない。この雑種は葉に綿毛を有するがその密度は *R. hirsutus* よりも小である。

雑種の形質には両親の中間型をしめすものも多く、中でも著しいものに次のものがあげられる。1) 葉身の形: この雑種の葉形は第 2 図に示す如く非常に変異に富み、しかもこの葉型は芽が

第 2 図 雑種葉型の変異



開筈発展する頃の季節と関係が深く、早春冬芽が開いて形成された葉は 3 出複葉で小型であり、往々側出小葉に欠刻を生じる。4, 5 月頃新しく生じた枝条に形成される葉は 3 出複葉であるが、両側の 2 小葉はそれぞれ一つの大欠刻によつて 2 片となり、葉の大きさは中型である。梅雨期より初夏にかけて生ずる枝条に形成される葉は大型で 5 出掌状複葉である。更に盛夏より初秋にかけて形成される葉は小型の 3 出複葉で、冬芽が展開した時に生

じた葉型によく似ている。この様に雑種の葉形は季節により異なるが、大体 3~5 出掌状複葉であるといえる。両親植物である *R. hirsutus* 及び *R. trifidus* の葉形は前者は 3 又は 5 出の羽状複葉であり、後者は 7 裂掌状単葉であるから、この雑種の葉形は複葉ではあるが大体両親の中間型と考へられる(第 3 図)。2) 赤色腺毛: 赤色腺毛は両親

第 3 図 両親及び雑種の葉型比較



左 *R. trifidus* 中央 hybrid 右 *R. hirsutus*

及び F_1 とも若い茎に存在するが第 1 表で見える如く形、長さ及び茎における分布等この F_1 は両親のはほぼ中間型をしめす。3) 以上の外托葉の形、雄蕊の花糸の長さ、等において雑種は両親の中間型をしめす。

稔性: 両親植物である *R. hirsutus* 及び *R. trifidus* では種子の稔性が第 2 表にしめすごとくである。即ち自然及び人工受粉を行つた場合いづれ

第 2 表 稔 性

植物名	交配方法	人工交配	自然交配
<i>R. trifidus</i>		74%	68%
<i>R. hirsutus</i>		83%	80%
hybrid		不 稔	不 稔

も 68% 以上で良好である。しかるに F_1 雑種はほとんど不稔であつて、ごく稀に果実をつけるものもあるが、1 聚果における果粒数は 1 乃至数個であり、果粒の大小は種々で安定していず、種子も微小で発芽力がない。

開花期: 松山市附近において *R. hirsutus* 及び *R. trifidus* の開花期は前者が 3 月上旬より 4 月下旬、後者が 4 月上旬より 5 月下旬に及んでいるが、この雑種は両親植物の何れに比しても開花

第 4 図 雑種の減数分裂



を見るが、 $2n=14$ の全部の染色体が 2 個染色体を形成する場合 (第 4 図, a) と 1 個染色体が 2 個及至数個あらわれる場合とがある。P. M. C. の第 1 分裂中期において、筆者の観察した 339 個の核板における染色体対合様式には $7II$, $6II+2I$, $5II+4I$, $4II+6I$ 及び $3II+8I$ があるが、これらのあらわれる頻度は第 3 表にしめすごとく雑種の個体及び調査を行つた年により相当の差異をしめす。即ち 1953 年に調査した個体では $5II+4I$ (第 4 図, d) のあらわれる頻度が最も高くして全体の 43% をしめ、又 1 年後の 1954 年に調査した個体では $7II$ (第 4 図, b) のものが最も多く現れ全体の 53% をしめている。この両者の合計からみると $6II+2I$ (第 4 図, c) の現れる頻度が最も高くして 39% をしめす。 $4II+6I$ 及びそれ以上に 1 個染色体の現れる場合は極めて少く、その率は 1% 内外である。減数第 1 分裂後期に染色体橋をみることがあるが (第 4 図, g) ごく稀である。中期において細胞内に分散していた 1 個染色体は後期に両極の染色体群の構成に入る場合と、両極の中間部に取残される場合 (第 4 図, e) とがある。後者の場合 1 個染色体は細胞内随意の位置に小核を形成する (第 4 図, f)。前者の場合、減数第 2 分裂中期に P. M. C. の両極に形成される核板の染色体数について、1953 年に観察した

第 3 表 雑種の減数分裂第 1 分裂中期における染色体対合

染色体対合型式		7II	6II+2I	5II+4I	4II+6I	3II+8I	合 計
調査年及び個体							
A	1953	23	81	83	3	2	192
B	1954	78	52	16	1	0	147
計		101	133	99	4	2	339

の時期が早く又終花の時期もおそい、即ち 1954 年の観察によると雑種は 2 月 14 日に開花し 6 月下旬より 7 月上旬にかけて残花が見られたから、開花期間は 4 ヶ月半という長期間である、この雑種は前述せる如く聚散花序をなすが、その着花数も両親植物に比して多い。

減数分裂: この雑種は体細胞で 14 個の染色体が算定されるから 2 倍体である。この雑種の花粉母細胞における減数分裂をみるとデアキネネス期において環状或は X 状に結合した 2 個染色体

76 個の P. M. C. では、染色体がそれぞれ 7 個と 7 個の場合 (第 4 図, i) が多くて 71% をしめ、残りの 20% は 8 個と 6 個の場合 (第 4 図, h) であつた。減数第 2 分裂中期以降においてしばしば P. M. C. の赤道面附近がヘマトキシリン液で濃染される状態となり (第 4 図, j), 更に時期が経つにつれてこの現象は細胞内全体にひろがる (第 4 図, k, l)。

花粉粒: この雑種は花粉の量が極めて少く、アセトウーミン染色によつてこれをしらべると内容

第 4 表 雑種の花粉内容

事 項 \ 花粉内容	空 虚	充 実	合 計
員 数	453	34	487
百 率 分	93%	7%	

第 5 表 雑種及び両親植物の花粉粒の大きさと頻度

種 類 \ 花粉粒の長径	18 μ 以下	18.1-23 μ	20.1-20 μ	22.1-24 μ	24.1-26 μ	26.1-28 μ	28.2-30 μ	30.1-32 μ	32.1-34 μ	34.1-36 μ	不定型	合 計
<i>R. trifidus</i>					3	5	31	70	16	14	9	148
<i>R. hirsutus</i>					2	4	29	70	27	12	7	151
hybrp	7	23	148	17	8	2	11	10	1	2	37	266

空虚なものと、充実しているものとがありその数は第 4 表の如く空虚なるものの数が内容の充実しているものよりも遙に多くて 93% をしめる。この雑種の花粉粒の大きさを両親植物のそれと比較したのが第 5 表である。第 5 表でみる如く両親植物の *R. hirsutus* 及び *R. trifidus* は花粉粒の長径が 24 μ から 36 μ の間にあつて 30 μ ~32 μ の花粉の現れる頻度が最も高い。雑種の花粉粒の大きさは両親に比して小さなものが多く、大小の変異の中も両親に比較して広い。即ち小なるものは花粉粒の長径 10 μ 程度であるが大なるものは 36 μ に及び、最も多く現れるのは長径 20 μ ~22 μ のものである。本雑種の花粉粒の大きさと員数の関係をしめす変異曲線は 2 頂をしめし、その一頂点は前述した如く 20 μ ~22 μ の所で高く、他の一つは 30 μ 前後の所で、前者に比較すると大変低い。後者の位置は両親植物における花粉粒の大きさの変異曲線の頂点の位置とほぼ一致する。この雑種の内容空虚な花粉はすべてみな小型の花粉である。

考 察

この雑種の両親植物である *R. hirsutus* 及び *R. trifidus* は 2 倍種で染色体数がいづれも 2n=14 である。その F₁ も染色体数が 2n=14 であるから、染色体数から見ると雑種の染色体は両親植物の各々の配偶子のもつ染色体の和で構成されていると考えられる。Koidzumi (1913) によると *R. hirsutus* (*R. Thunbergi* Sieb. et Zucc.) と *R. trifidus* は分類学上所属する *Subgenus* が異り、

前者は *Subgenus* *Idaeobatus* Focke に属し、後者は *Subgenus* *Anopleobatus* Focke に属する。*R. hirsutus* 及び *R. trifidus* は前述せる如く外部形態にも大分差異があり、又前者は草木状、後者は灌木状である。しかるにこの両者は容易に雑種を生じ、種子の発芽及び発芽後の生育状態は、筆

者が現在迄に作つたキイチゴ属植物の種間雑種のどれよりも良好であつた。即ち筆者の行つたキイチゴ属の種間雑種では、その組合せによつては往々発芽後白化現象を起して枯死するものや、发育の極めて悪いものを生じるが、この雑種では発芽及び发育に対する障害は全くなく生育は甚だ良好である。

この雑種では無性繁殖力、花の大きさ、花序における着花数等両親植物の何れのものよりも優れている形質があるが、これは両親の形質が交雑することによつて雑種強勢の現象をしめしたものと考へられる。この雑種の葉の大きさ及び形は前述した如く時期によつて大変異つている。両親植物の葉も *R. hirsutus* は羽状複葉であり、*R. trifidus* は掌状単葉で両者は形態的にも差異が極めて大きく、この両者の交雑によつて生じた雑種は葉の形質に関し複雑な遺伝子構成をもつものと考えられる。雑種の葉形の変異の方向が季節によりほぼ一定していることは、遺伝子の表現に環境要因が強く働きかけるものと思れる。

Thomas (1940) は *Rubus* 属数種の種間雑種で花粉母細胞の成熟分裂第 1 分裂中期における染色体の対合状態にもとづいて供試材料のゲノム式を決定しているが、それらの中には我国産のものはない。この雑種の成熟分裂第 1 分裂中期における染色体対合状態をみると、7II, 6II+2I 及び 5II+4I の現れる頻度は何れも高く 29% 以上であるが、1 価染色体が 6 個以上現れる場合の頻度は極めて少い。このことからして雑種の両親である

R. hirsutus 及び *R. trifidus* のゲノムを構成するそれぞれ7個の染色体中5対の染色体は親和性が強く、残りの2対の染色体は前者に比して親和性が強いものと考えられる。この雑種はほとんど果実を形成せず稀に一果に1~数個の果粒をつける程度でほとんど不稔性である。これは両親植物のゲノムを構成するそれぞれ7染色体中一部染色体

の不親和によることと、更に配偶子形成の過程において、途中一部の花粉母細胞の内容が変質するため良好の配偶子が数多く得られないこと等が原因の一つと思われる。

最後にあたり種々御指導を頂いた恩師下斗米教授に厚く感謝の意を表する。

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Resume

1. This paper deals with the results of the research on the characteristics and type of pairing of chromosomes in the reduction division in P.M.C. of the F_1 -hybrid which is raised artificially by crossing *Rubus hirsutus* Thunb. (♂) and *R. trifidus* Thunb. (♀).

2. The seeds obtained in the cross are easily germinated. The growth of the F_1 -hybrid is well.

3. The types and frequency of the pairing of the chromosomes in the metaphase of first division in the hybrid P.M.C. are as follows: $7_{II} \dots 30\%$, $6_{II}+2_I \dots 39\%$, $5_{II}+4_I \dots 29\%$, $4_{II}+6_I$ and $3_{II}+8_I \dots$ less than 1%.

4. The 93% of pollen of this hybrid is empty and the rest is full in content. The seeds of this hybrid are sterile.

5. This hybrid does asexual propagation vigorously. The larger size of the flowers and more numerous in flower-cense of the hybrid as compared with those of the parents indicate that it shows heterosis.

本 会 記 事

このたび新しい編集委員として次の 16 名が指名されました。

小林義雄, 佐竹義輔, 正宗嚴敬, 亙理俊次,
田中信徳, 小野記彦, 芳賀 恣, 新家浪雄,
大槻虎男, 渡辺清彦, 長谷川正男, 下郡山正巳,
長尾昌之, 森 健志, 宇佐美正一郎, 宝月欣二,
門司正三,

支 部 通 信

中 部 支 部

第 3 回大会。5 月 8 日(日)三重県立大学水産学部において, 講演 (1) 川松重信: ミトリササが種子の発芽に伴う各器官の澱粉の消長について, (2) 高木典雄: 蘚類の一新属 *Pseudopheuropus* について, (3) 倉内一二: 新田植物群落の海水侵入に対する抵抗(続), (4) 熊沢正夫: ヒガンバナ科鱗茎の分岐法(予報), (5) 毛利定弘・森 隆也: いんげん豆の発芽時に於ける炭水化物の変化について, (6) 滝川 満・森 隆也: バラ科植物花粉に関する研究, (7) 伊藤道夫: モエジマシダの前葉体の反転について, (8) 井沢三生: シロバナタンポポの花軸の生長と生長物質との関係について, (9) 瀬木紀男・後藤和四郎: 志摩半島沿岸潮間帯の海藻について, (10) 加藤幸雄: シダの細胞分化に関する二・三の知見, (11) 岩崎秀一・森 健志: 細胞の細胞外抽出液による窒素放出反応, (12) 須賀 英文: *Tolypella gracilis* Imahori (Characeae) の年週期と生態について(予報), (13) 原田市太郎: カワツルモの細胞形態学, (14) 谷口森俊: 伊勢湾沿岸の暖地性植物の分布, (15) 高橋千裕: スギナの胞子は生活しているということ, (16) 瀬木紀男: アサクサノリ, (17) 神谷平: 淡水藻類の生態的分類の試み, (18) 矢頭献一: 南伊勢山地の植物, 特別講演。吉井義次: 生活形と生態型。

新 入 会 (30 年 4 月)

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編集後記

去る5月19日新委員による第一回の編集会議があり6月並びに7月号に掲載する論文を決定致しました

6月号掲載論文

受付番号, No. 20 (受付月日, 2. 22) 栗田正秀:
キンボウ科の細胞学研究 II. イチリンソウ属
ユキワリソウ属の核型 (和文)

No. 21 (2. 27) 石川茂雄: 発芽の光感性 II.
イワアカバナの光期と暗期との相互関係 (英文)

No. 22 (2. 28) 増田芳雄: 原形質の溶質透過性
に対するオーキシンの影響 II. (独文)

No. 26 (3. 19) 矢野孝二: 薔類の染色体 IX.
ハイゴケ属の核型, 性染色体及倍数性 (和文)

No. 29 (3. 28) 高見亘: アルカリ土金属の蓚
酸塩の晶癖とその植物学的意義 (和文)

7月号掲載予定

No. 14 (2. 2) 伊倉伊三美: シダ類の配偶体
IX (2) (3) (英文)

No. 31 (4. 5) 高原・川名・丹下: 受光量と土壤
水分の量とガシラガシ苗の耐陰性におよぼす影響
(和文)

No. 33 (4. 9) 広江美之助: パセリーとセルリ
ーの細胞分類学的比較 (英文)

No. 37 (4. 13) 米山穰: 松蜜から分離した酵
母菌 I-(1) (英文)

No. 38 (4. 30) 鳥山英雄: 感覚植物の研究 V.
(英文)

No. 37 (4. 13) 米山穰: 松蜜から分離した酵
母菌 I-(2) (英文)

No. 39 (4. 30) 中沢信午: スギモクの異常卵
(英文)

No. 41 (5. 7) Raman, V. S. インド産ジャス
ミンの細胞遺伝 I. 形態分類 (英文)

No. 42 (5. 18) 矢野孝二: 薔類の染色体 X.
タマゴケ科の核型と性染色体 (和文)

以上 10 編であります。

尙, 掲載未決定の現在受付中の論文は下記の
10 編であります。

受付番号 No. 15 (受付月日 2. 2) 伊倉伊三美:
シダの配偶体の細胞形態学的研究 IX (4) (英文)

No. 30 (4. 2) 小野林・小長光与壮: 遊離葉緑体
における澱粉形成 (英文)

No. 32 (4. 9) 沢村正五: 有絲分裂の生体観察
に利用できる植物材料 (和文)

No. 34 (4. 12) 瀬川宗吉: 有節サンゴモの解剖
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No. 35 (4. 12) 鈴木米三: ハシリドコロのポリ
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いて (英文)

No. 36 (4. 12) 同上 II. DOPA の酸化につ
いて (英文)

No. 39 (4. 3) 中沢信午: スギモクの異常卵
(英文)

No. 41 (5. 7) Raman, V. S.: インド産ジャス
ミンの細胞遺伝学 I. 形態, 分類 (英文)

No. 42 (5. 18) 矢野孝二: 薔類数種の染色体
X. タマゴケ科の核型と性染色体 (和文)
(石川茂雄)

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Light-sensitivity against the Germination II.*

Interaction of Light and Darkness on the Germination of *Epilobium cephalostigma* Seeds

by Sigeo ISIKAWA**

石川茂雄: 発芽の光感性 II. イワアカバナの種子の発芽に際しての光と暗との相互作用

Received February 27, 1955

Introduction

The author referred in his precedent report¹⁾ that what is called "photoperiodism" could be recognized in germination of light-favoured seeds. It means that, by subjecting these seeds to daily light exposure, one can find the optimum length of light period for germination of each kind of seeds respectively and that germination rates go down positively under continuous illuminating in most kinds of seeds. From this, an existence of a certain length of dark period after initial light period was suggested to be required for complete germination of light-favoured seeds.

The present paper reports what has been studied about the germination of *Epilobium* seeds; (1) the existence of two light periods and an intermediate dark period of a certain duration, all of which were necessarily required for the germination, and (2) individual character of the two light periods and the one dark period and other facts studied on them.

The writer wishes to express his cordial thanks to Professor Sizuo Hattori and Dr. Masao Hasegawa for their kind guidances and valuable advices. Kind and precious assistance was extended to the writer by Mr. Takasi Yamazaki of Tokyo University in the determination of species and Mr. Takeshi Oofusa of Tokyo Metropolitan University throughout the experiments, to whom he also wishes to extend his heartiest thanks.

Experimental Methods and Materials

The experimental methods were almost the same as those employed in author's preceding study with the exception of the following points: (1) Presoaking time before exposure to light was 7 days. (2) Soaking time (in which the seeds were placed in darkness after the last illuminating) was 5 days. (3) In case illumination was performed at a low temperature, seeds were maintained before exposure to light in a low-temperature-apparatus for 30 minutes, during which the temperature of culture fluid and seeds themselves were lowered sufficiently, after then

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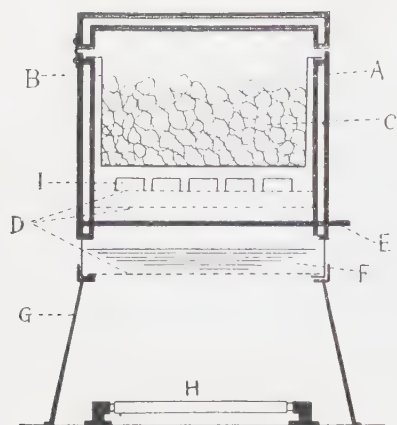


Fig. 1. Low-temperature-apparatus
A: box, B: tin-box, C: freezing mixture,
D: glass, E: shutter, F: water, G: legs,
H: light, I: experimented dishes

they were subjected to illumination as being kept in the low-temperature-apparatus (see Fig. 1).

The whole experiments were performed during the period from December, 1952 to January, 1955, which could be divided into three stages. A series of data recorded in the present paper was obtained from the results of the second experiment stage, which lasted from Dec., 1953 to July, 1954, for which the seeds collected at Mt. Akagi in Autumn of 1953 were used. Although the germination percentage obtained from the seeds of 1953 was very low (50-60 %) as compared with the same of 1952 (80-90 %), no change was observed in general tendencies of the germination throughout the experiments.

Out of the seeds studied, those for the first stage were collected in October, 1952 at Mt. Akagi, and those for the second, in October, 1953 at the same place, while the seeds used for the third experimental stage were gathered in October, 1954 at Mt. Nyugasa.

Experimental Results

Preliminary Experiment: The seeds used in the year of 1953 showed a comparatively low germination rate, but their optimum photoperiod for germination was of a rather wide range (6-21 hours), and lowering of germination rates was recognized under continuous exposure to light (Fig. 2-A). Thus, the seeds of 1953 showed, in general, the same tendency in respect of germination behavior as the seeds of 1952. Illuminating must be necessarily given then the degree of light sensitivity of seeds has been highered up sufficiently⁵⁾, and it was confirmed that the highest light sensitivity was maintained with a presoaking time of 6-12 days, in view of a tendency of light sensitivity of seeds which changes with the length of presoaking time (Fig. 2-B). For these reasons, presoaking time for the successive experiments was decided to be 7 days.

Experiment 1: It was revealed from the above experiment as shown in Fig. 2-A that the maximum germination percentage was obtained from 7 illuminations repeatedly performed over seven days (6 hours per day). And this time, in order to know how many times out of these seven illuminations is necessarily required for germination, the seeds were exposed 1~5 times to a light of 10^3 lux intensity for each 6 hours in 24 hour-cycle (for 1~5 days and 6 hours a day) when the light

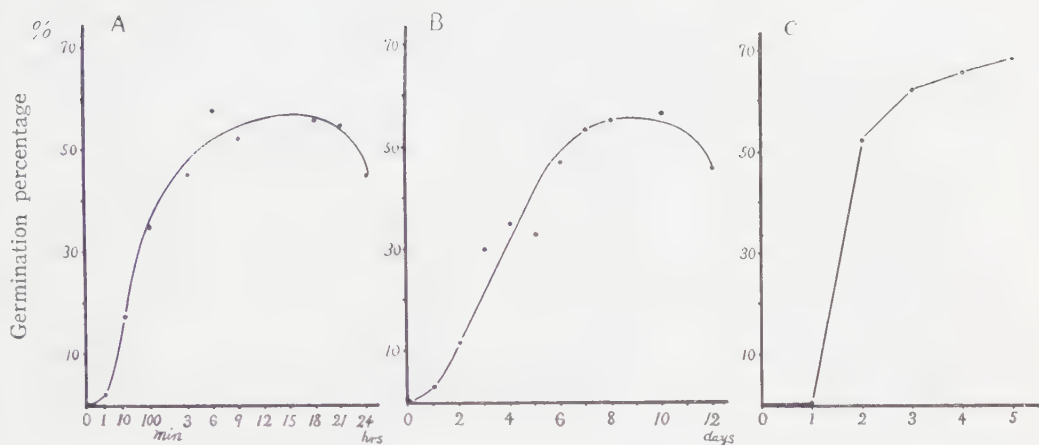


Fig. 2. A: Effect of the daylength, B: Changes of degrees of light-sensitivity with the lapse of presoaking time, C: Germination percentages of illuminating times

sensitivity mounted highly enough after seven presoaking days. Then the germination rates were counted (Fig. 2-C), and it was recognized that the highest germination percentage was attained by two times illumination of 6 hours in 24 hour-cycle. (It means illumination over two days, and 6 hours a day.)

Experiment 2: From the Experiment 1, and as described partly in the same item, it was further recognized that no germination was obtained from one time illumination lasting 6 hours, but only two times of 6 hours' illumination in 24 hour-cycle induced germination. Then, for this

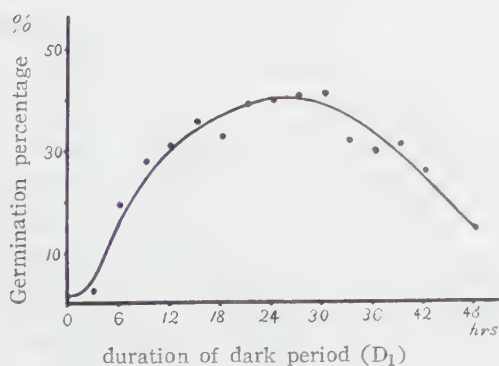


Fig. 3. Effect of the length of dark period (D_1) inserted between two illuminating durations each composed of three hours

time, one duration of 6 hours' light period (it did not induce germination) was divided into two illuminating durations each composed of three hours, between which one duration of dark period (D_1) of 0~48 hours was inserted. Then it was revealed that, out of every results from 0~48 hours, the maximum germination percentage was secured in 15~30 hours of an intermediate dark period (D_1) (Fig. 3). In case the dark period is short (0~6 hours) or too long (more than 42 hours), the rates of germination dropped remarkably.

Experiment 3: From the aforementioned experiments, the author could find two light periods with an intermediate dark period (D_1) of suitable duration (about 21 hours at 22°C) to be essential conditions inducing germination of *Epilobium* seeds.

Then, under these conditions, the author attempt to put various lengths of illuminating time into the first light period (hereinafter referred as L_1) and the second light period (hereinafter referred as L_2) respectively.

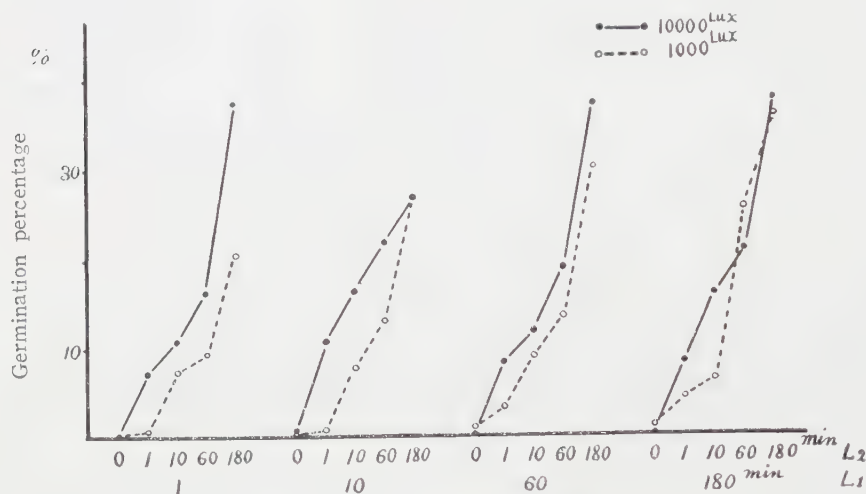


Fig. 4. Effect of two light periods of different duration

According to the experimental results given in Fig. 4, the germination rates did not change with the length of L_1 varying from 1 minute to 180 minutes, namely the rates were not affected by illuminating length of L_1 within this limit. Accordingly, it could be considered that an illuminating time as short as one minute might be enough for the first light process.

On the other hand, in L_2 , the longer illuminating time become, the higher germination rates were obtained, and it took 10^3 lux intensity of 3 hours' illuminating to obtain the highest rate.

Experiment 4: In the preceding experiment, 10^3 lux \times 1 min was recognized to be an enough length for L_1 . Next, the

author examined the germination values obtained from the sum multiplied light quantity (light intensity \times illuminating lengths) of two different intensities (10^2 lux and 10^3 lux) by various lengths (Fig. 5), and derived the following results: (1) the germination rates were not affected by light intensity but by multiplied light quantity. (2) 9×10^4 MKS was necessary and sufficient quantity.

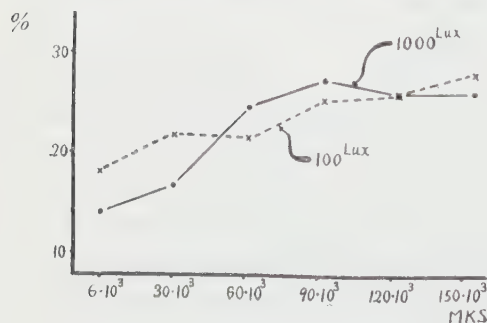


Fig. 5. Effect of light quantity required for the first illumination (L_1)

Afterwards, a sufficient multiplied light quantity (120×10^4 MKS or 2 minutes' illuminating with 10^4 lux) was given to seeds in L_1 and then in L_2 , the seeds were

subjected separately 10^3 lux and 10^4 lux intensities with various. Then the germination percentages were counted (Fig. 6). They were affected by multiplied light quantity rather than the length of light period, so it might be considered that illuminating length was an important factor for germination in L_2 .

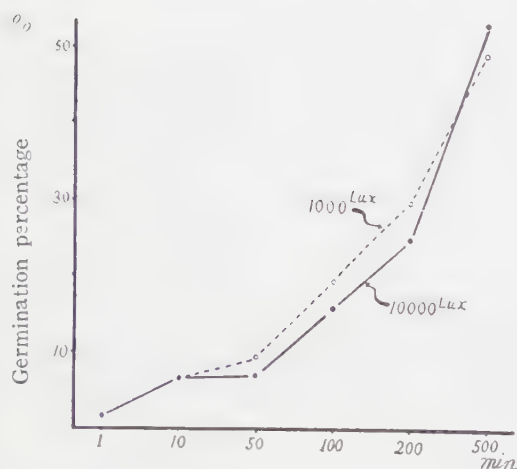


Fig. 6. Effect of the second illumination (L_2) of different duration

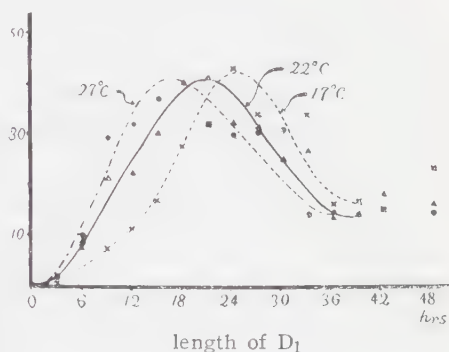


Fig. 7. Effect of the temperature of dark period (D_1) inserted between two periods of illumination

Experiment 5: Next, studies were made on the length of dark period (D_1) inserted between L_1 (short) and L_2 (long), and how the optimum length of dark period (D_1) did change with fluctuation of temperature was investigated (Fig. 7). In consequence, the author could find that the length of dark period had a close relation to temperature.

Experiment 6: The next experiments were made under the basic condition already proved, that is, a short illumination was given for L_1 , a long illumination for L_2 and the length of dark period (D_1) was settled for 21 hours at 22°C . Then, individually different low temperature were given for the two light periods (L_1 and L_2), and the germination percentages were counted. Table 1 is a comparison of the

Table 1. Effect of temperature during L_1 of $2000 \text{ lux} \times 10 \text{ min}$

Temperature ($^\circ\text{C}$)	22	-2~0
Germination percentage	33.8%	30.8%

L_2 : $2000 \text{ lux} \times 180 \text{ min}$, presoaking period: 7 days, soaking period: 5 days, D_1 : 21 hrs

germination rates obtained from the illumination at 22°C for L_2 and at a low temperature ($0\sim 2^\circ\text{C}$) for L_1 , with those from the illumination at 22°C for both periods. (In this case, temperature was changed only for L_1). Any difference of the rates could not be found between the two cases. Thus, it was understood that L_1 is not affected by temperature. Table 2 is a comparison of the germination

Table 2. Effect of temperature during L_2 of 2000 lux \times 180 min

Temperature ($^{\circ}\text{C}$)	22	-2~0
Germination percentages	29.6%	1.2%

L_1 : 2000 lux \times 10 min, presoaking period: 7days, soaking period: 5 days,
 D_1 : 21 hrs.

rates from the illumination at 22°C for L_1 and at a low temperature ($0\sim 2^{\circ}\text{C}$) for L_2 , with those from the illumination at 22°C for both periods. (In this case, temperature was changed only for L_2). This time, few germination was seen in case illumination for L_2 was performed at low temperature.

Considerations

In the present work, the author investigated and confirmed that two times of illumination and a certain length of dark period between the two light periods are required for the germination of *Epilobium* seeds. For L_1 , only a short time illumination is required and the multiplied light quantity required for the same period is about 9×10^4 MKS, and the germination rate is not affected by temperature. From this, the first light period which promotes the germination of *Epilobium* seeds should be considered as a single photochemical reaction of low intensity process.

Seeds of Tobacco, lettuce²⁾ and *Lythrum Salicaria*⁶⁾ germinate completely by one time short exposure to light. The values of the required multiplied light quantity of these seeds are generally similar to the same of *Epilobium* seeds. Action spectrum of seed germination of lettuce (by Borthwick¹⁾ and others) resembles to be the same of low intensity process in floral initiation. From these facts, it can be considered that what is seen on germination behavior of *Epilobium* in L_1 may be of a similar nature to the above phenomena. A conclusion, however, cannot be reached as yet. The author considers that further investigations of L_1 by action spectrum is desirable and must be performed, and he expects to do so.

L_2 requires long illumination of high intensity and is affected by temperature. Therefore, it can be considered that not only a single photochemical reaction but also enzymatic reaction have influence on germination behavior in L_2 . But the author could not find any actual indications yet. As already described, an existence of a dark period (D_1) of a certain length was confirmed to be necessary for germination of *Epilobium* seeds. But, in the present investigation it was not confirmed whether light hinders dark reaction as the case of the floral initiation of Short Day plant, nor measured reaction speed in dark period (D_1).

Many reports have been presented recently, which confirm that a short time or a momentary illumination³⁾ is quite enough for promoting the germination of tobacco seeds. In the meantime, the author has treated the seeds of *Lysimachia mauritiana*,

Oenothera parviflora, *Rumex* sp., *Silene Armeria* as well as tobacco, all of which can germinate completely by one time short illumination, and obtained without fail the highest germination percentages at a low temperature (0~2°C) (Table 3).

Table 3. Effect of temperature during the illumination of 2000 lux×1 min

Species \ Temp. (°C)	23	-2~0
<i>Oenothera parviflora</i>	69.3%	66.7%
<i>Lysimachia mauritiana</i>	54.3	41.0
<i>Rumex</i> sp.	78.0	78.7
<i>Silene Armeria</i>	95.0	96.0
<i>Nicotiana Tabacum</i>	75.7	76.7

From these records, the author considers that the report⁷⁾ on tobacco germination issued in 1954 by Ogawara regarding his recognition of two light periods with an intermediate dark period as fundamental requirements for seed germination of tobacco ought to be reexamined.

Conclusions

The author recognized that in order to induce germination of *Epilobium cephalostigma* seeds, one of the light-favoured seeds, two light periods and one dark period (D_1) of a certain length between the two are required. As the multiplied light quantity required for L_1 is no more than 9×10^4 MKS, L_1 can be ended with one minute illumination of 10^3 lux and is not affected by temperature.

L_2 requires strong intensity (10^3 – 10^4 lux) and long exposure to light (more than 200 minutes and for the maximum percentage, 500 minutes), and it is affected by temperature. Therefore, the second light period L_2 is considered as a complex high intensity process connecting not only with a single photochemical reaction but also with enzymatic reaction. As the length of dark period (D_1) between the two light periods is in inverse proportion to temperature, affection of dark period on germination is considered as enzymatic reaction. However, it has not yet been confirmed whether this dark period reaction is hindered or not.

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Über den Einfluss von Auxin auf die Stoffpermeabilität des Protoplasmas

II. Mitteilung. Harnstoffpermeabilität des Protoplasmas von *Avena*-Koleoptile

von Yoshio MASUDA*

増田芳雄: 原形質の溶質透過性に対するオーキシンの影響 II.

Eingegangen am 28. Februar 1955

In einer vorigen Mitteilung hat der Verfasser gezeigt, dass die Permeabilität der Zwiebschuppenepidermiszellen von *Allium cepa* für Harnstoff und Glycerin durch das Heteroauxin (H. A.) deutlich beeinflusst wird (Masuda, 1953). Dabei zog er auch den Schluss, dass das H. A. auch auf die Lipoidphase der Protoplasamembran, wie auf die Eiweissphase, wirkt.

Nun kommt die Frage, ob (A) das Auxin, das sich spontan in der Pflanzenzelle befindet, auf die Permeabilität einen Einfluss ausübt, und ob (B) es irgendeine Beziehung zwischen der Permeabilitätssteigerung und der Zellstreckung gibt. Davon berichtet der Verfasser folgende Versuchsergebnisse.

Material und Methode

Als Versuchsmaterial wurden die Innenepidermiszellen der etiolierten *Avena*-Koleoptile gewählt. In den Versuchen, um die Zellen, deren Gehalte des in ihnen befindlichen Auxins verschieden sind, herzustellen, wurden die obere und untere Hälfte der etiolierten normalen und dekapitierten Koleoptilen angewandt.

Die Permeabilitätsbestimmung wurde nach der Deplasmolysezeit-Methode gemacht. Dafür folgte der Verfasser derselben Methode, die in der hängenden Tropfen-Kultur-Methode von Takada (1953) gebraucht wurde, um die Konzentration des Plasmolytikums konstant zu halten und den Gasaustausch zu gewähren.

Die Plasmolyse-Deplasmolyseverläufe wurden mit dem Prozent der plasmolysierten Zellen gegen die Gesamtzahl eines mikroskopischen Feldes ausgedrückt. Und wenn ihr Wert weniger als 5 erreichte, wurde die Zeit als Deplasmolysezeit angesehen (Masuda, 1953). Alle Versuche wurden bei 25–26°C ausgeführt.

Um den Effekt des Heteroauxins für die Streckungen der Koleoptilen zu bestimmen, wurden die aus den 20–30 mm langen Koleoptilen ausgeschnittenen 4 mm langen Zylinderstücke in die Heteroauxinlösungen von verschiedenen Konzentrationen oder

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ins bidest. Wasser gebracht, und ihre Längen wurden in der dunklen Stelle in allen 6 Stunden gemessen.

Versuchsergebnisse

(A) Spontanes Auxin und Harnstoffpermeabilität.

Als Objekt wurden die junge 20-30 mm lange Koleoptilen und die 50-60 mm lange, die die Streckung geendet haben, angewandt. Wenn man sie dekapitierte oder nicht, teilte man sie jede für sich in die obere und untere Hälfte ein. Nach der Wässerung wurden diese Koleoptilenstücke in die 0.7 M Harnstofflösung gebracht.

Die gemessenen Deplasmolysezeiten werden in Tabelle 1 gezeigt. Diese Werte sind gross in den Zellen der Koleoptilen, die fast die Streckung geendet haben, und klein in den der heranwachsenden. Sie sind bei den normalen Koleoptilen kleiner als bei den dekapitierten, und kleiner auch bei der oberen Hälfte als bei der unteren.

Tabelle 1. Deplasmolysezeit in der 0.7 M Harnstofflösung der Zellen der verschiedenen Wachstumszustände

	Koleoptilenlänge			
	20-30 mm		50-60 mm	
	Obere	Untere	Obere	Untere
Normal	15-20 min.	30-35	90-100	90-100
Dekapitiert	30-35	35-40	90-100	90-100

Hier kommen die osmotischen Werte und die Plasmaviskosität der Zellen, deren Wachstumsprozesse verschieden sind, in Frage. Aber nach der Grenzplasmolyse-Methode belaufen sich die osmotischen Werte in allen gebrauchten Zellen fast auf gleiche Werte (etwa 0.5 M Glukose). Auch die Viskosität ist nach Plasmolysezeit fast gleich. Also werden die gemessenen Deplasmolysezeiten als das allgemeine Mass der Harnstoffpermeabilität Betrachtet, und daraus kann man sich denken, dass die Harnstoffpermeabilität der Zellen, die die Streckung geendet haben und nur wenig spontanes Auxin enthalten, niedrig ist und die der heranwachsenen Zellen, die viel spontanes Auxin haben, hoch ist.

(B) Zellstreckung und Harnstoffpermeabilität.

Die Beziehung zwischen der Streckung der Koleoptilen und der Konzentration des H. A. wird in Abbildung gezeigt. Wenn die Koleoptilenstücke nur mit dem H. A. versorgt wurden, streckten sie sich zuerst etwa im Verhältnis zur Konzentrationszunahme des H. A., aber schon nach 6 Stunden stand die Streckung fast still. Wenn 3 % Rohrzucker, ausser dem H. A., ins Medium hinzugesetzt wurde (McRae und Bonner, 1953), dauerte die Streckung lange Zeit, so daß der Effekt des H. A. für die Streckung der Koleoptilenstücke sehr klar in die Erscheinung trat.

Wenn ausser dem H. A. der Zucker nicht hinzugesetzt wurde, wie die Tabelle 2

Tabelle 2. Deplasmolysezeit der Koleoptilenzellen, denen nur. H. A. gegeben wurden, in der 0.7 M Harnstofflösung

	3 Std.	6 Std.	9 Std.	12 Std.	24 Std.
Kontrolle	35-40 min.	40-45	45-50	50-55	60-65
0.1 mg/1 H. A.	35-40	35-40	45-50	60-65	95-100
1 mg/1 H. A.	30-35	35-40	50-55	70-75	110-115

Tabelle 3. Deplosmolysezeit der Koleoptilenzellen, denen H. A. und 3 % Rohrzucker gegeben wurden, in der 0.7 M Harnstofflösung

	3 Std.	6 Std.	9 Std.	12 Std.	24 Std.
Kontrolle	25-30 min.	25-30	20-25	plasmoly-	nicht
0.1 mg/1 H. A.	20-25	20-25	17-20	siert nur.	plasmoly-
1 mg/1 H. A.	15-20	15-20	12-15	wenig.	siert.

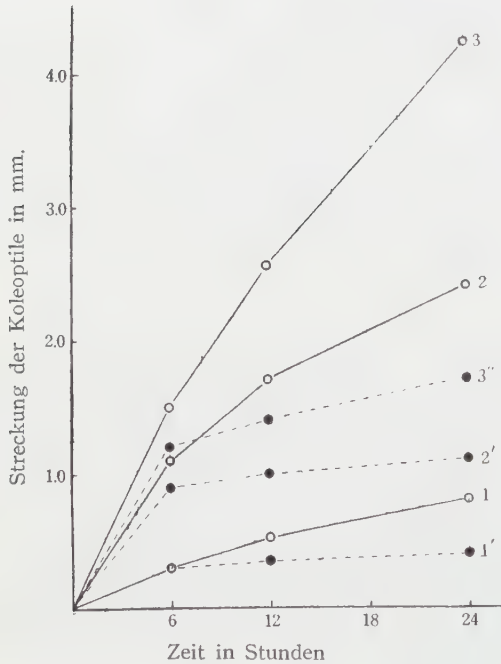


Abb.: Die Streckungsverläufe der Koleoptile, die nur H. A. (●.....●) und ausserdem 3 % Rohrzucker (○—○) gegeben wird. Anfängliche pH 6.6-7.0. 25° C. 1 und 1': Kontrolle, 2 und 2'; 0.1 mg/1 H. A., 3 und 3': 1 mg/1 H. A..

zeigt, nahm auch die Harnstoffpermeabilität im Verlauf der Zeit ab. Wenn aber der Zucker hinzugesetzt wurde, wie die Tabellen 3 und 4 zeigen, fiel sie 24 Stunden lang nicht. Wenn die Koleoptilenzylinder in der 3 % Zuckerlösung (0.08 M) 12 Stunden lang gebadet wurden, nahm der osmotische Druck der Koleoptilenzellen zu, wenn man auch das H. A. hinzusetzte oder nicht, und plasmolysierten sie nicht in 0.7 M Harnstofflösung plasmolysieren (Tabelle 3). Daher mussten die Versuche in 0.8 M Harnstofflösungen ausgeführt werden.

Besprechung

Nach dem Versuche A kann man erkennen, dass die Harnstoffpermeabilität und die Streckung der Koleoptilen, die das spontane Auxin vielleicht verursacht, parallel sind.

In dem Versuche B, bei Zusatz von 3 % Rohrzucker wurde die Permeabilität, wie die Streckung, mit den Konzentrationszunahmen des H. A. vermehrt, und ohne Zusatz des Zuckers, fiel die Harnstoffpermeabilität wie die Streckung schon nach einigen Stunden herab.

Tabelle 4. Deplasmolysezeit der Koleoptilenzellen, denen H. A. und 3 % Rohrzucker gegeben wurden, in der 0.8 M Harnstofflösung.

	3 Std.	6 Std.	9 Std.	12 Std.	24 Std.
Kontrolle	25-30 min.	25-28	18-22	18-22	15-18
0.1 mg/1 H. A.	20-25	20-25	15-20	15-18	13-16
1 mg/1 H. A.	15-18	15-17	13-16	13-15	12-15

Wie oben gesagt, kann man jedenfalls erkennen, dass Harnstoffpermeabilität und Streckung parallel sind. Da die Permeabilität nach der Deplasmolysezeit-Methode gemessen wurde, kann man in diesem Falle verstehen, dass der Zucker die Kolloidzustände der Plasmagrenzschicht eher beeinflusst als er die Kohlenstoffquelle der aktiven energieverbrauchenden Stoffaufnahme ist. Aber nach den berichteten Versuchsergebnissen allein, wie Guttenberg und Beythien (1951) gezeigt haben, kann man nicht entscheiden, ob die durch das Auxin hervorgerufene Permeabilitätserhöhung auf der Streckungserhöhung beruht, oder ob die Plasmazustände, wenn sich das Auxin und die nutzbare Kohlenstoffquelle befinden und infolgedessen die Streckung gefördert wird, die Permeabilitätserhöhung usw. verursachen.

Zusammenfassung

1. Die Harnstoffpermeabilität der Innenepidermiszellen von etiolierten *Avena*-Koleoptile wurde nach der Deplasmolysezeit-Methode gemessen.

2. Wenn die verschiedenen Bedingungen der Streckung von *Avena*-Koleoptilenstücke, d.h. das Alter und die Dekapitation der Koleoptile, und Zusatz von Heteroauxin und Zucker, verändert wurden, wurde die Tendenz gefunden, dass die Streckung und die Harnstoffpermeabilität parallel sind.

Hier sei es dem Verfasser gestattet, seinem verehrten Lehrer, Herrn Prof. Joji Ashida, für seine Anregung und ständige Anleitung den besten Dank auszusprechen.

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Cytological and Morphological Studies on the Gametophytes of Ferns IX The Polar Plasmolysis on Fern-prothallium (2)*

by Isami IGURA**

伊倉伊三美: 羊齒類の配偶体に関する細胞学的並に形態学的研究 IX
羊齒類前葉体の有極性原形質分離 (2)

Received February 2, 1955

From the results shown in Table 1 in the preceding paper, the following facts are explained. The plasmolyses of the prothallial cells occurred, first of all, at Region I and extended by degrees to II, III, IV, V and VI but occurred often almost at the same time at I and II. Accordingly, the plasmolytic gradient was observed among the regions in the whole field of the prothallium, and the basal pole produces the plasmolysis earlier than the apical one. The spot at which the cytoplasm separates from the membrane firstly in a single prothallial cell was regarded as the corner of the apical pole in many cases (Photo. I), while sometimes the cases which were contrary to this fact were observed. That is to say, in a single cell, the plasmolysis is generally positive, at the beginning, in the corner of the cell at the apical pole and negative at the basal one, and the cytoplasm is apt to draw near the latter. The reverse phenomenon of this behaviour of the cytoplasm, however, was observed in some portions. Namely, in a certain cell the cytoplasm separated from the membrane at the corner of the cell at the basal pole and gradually reached A-type.

The region which reached, at first, the A-type of the form of plasmolysis in the prothallial cell was I also, and the other regions followed I one after another. In the same mol.-solution and the same duration each region showed the different type, that is, though Region I and II presented A-type, the other regions did not get A-type and remained at B- or C-type (Photos. 2, 3, 4-6, and 7-9). B- and C-type changed their forms by degrees into A- or B-type, at last after some times, different as the time was according to the species of the prothallia or the concentration of mol.-solution, all regions got A-types (Photos 10, 11). In the prothallial cell which seemed to be divided into two cells by the transversal membrane, the negative plasmolysis was observed at the both sides of this transversal membrane in spite of the cell polarity (Photo. 7), and the opposite sides to this membrane showed the incipient plasmolysis. This result may be due to the fact that the cytoplasm contacts

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Table II. The duration of plasmolysis at each region in the whole field of the prothallium

Plasmolytica	Species	Mol.	Regions						Remarks	
			I	II	III	IV	V	VI	date	temp. °C
Saccharose	Ai	II, III 0.30	(mins.)						II-6	12, 7
		0.30	80-90	90-110	110-120	110-120	110	130		
		0.30	110-120	115-120	120-140	120-140	140	150		
	Lm	II 0.30	90-100	90-100	100-110	100	120	100 130	II-24	15, 11 5
		IV-V 0.40	80-90	80-100	90-100	100-110				
		0.20	50-60	50-60	60-50	60-65				
CaCl ₂	Ai	V 0.30	60-65	60-65	65-75	70-90			II-5	17, 8.5
		0.50	30-35	30-50	60-65	70-80	70-80			
		V 0.30	50-60	50-60	50-70	60-70	70-80			
	Lm	III IV 0.18	50-60	50-60	50-70	60-70?	60-70?		II-20	19.5, 12
		IV-V 0.30	30-35	30-35	35-50	65-75	75-100?			
		0.30	120-150	120-150	120-160	140-160				
Urea	Ai	II 0.42	60-70	60-80	70-80	70-80	80-90		III-2	16, 9
		V 0.70	20-25	20-25	30-40	40-50	50-60			
		1.00	15-20	15-20	20-30	40-50	40-50			
	Tj	IV 0.40	60-70	60-70	70-80				III-8	20, 10
		0.70	15-20	15-20	30-40	40-50	40-50			
		IV-V 0.38	40-50	40-50	50-60	50-60				
KCl	Ai	V-VI 0.58	30-40	30-40	40-50	50-60	60-70		III-10	19.5, 12
		V-VI 0.62	20-30	20-30	30-40	40-50	50-60			
		V 0.30	30-40	30-40	40-50	60-70	70-80			
	Tj	1.00	20-30	20-30	30-40	40-50	60-70	70-80?	III-12	19, 12
		V 0.30	20-30	20-30	30-40	40-50	40-50			
		V 0.30	15-20	15-20	20-30	30-40	40-50	40-50?		
KNO ₃	Ai	1.00	5-10	5-10	10-15	10-15	15-20	15-20?	III-16	16, 11.5
		V 0.28	15-25	15-25	20-30	30-40				
		IV 0.40	30-40	30-40	40-50	40-60	40-60			
	Tj	IV 0.40	15-20	15-20	20-30	30-40	50-60		III-17	17.5, 11
		IV 0.40	30-40	30-40	40-50	40-60	40-60			
		IV 0.40	15-20	15-20	20-30	30-40	50-60			
Glucose	Ai	IV 0.30	20-25	20-25	30-50	50-60			IV-21	18, 13.5
		1.00	10-20	10-20	30-30	30-50	40-60			
		IV 0.36	30-40	30-40	40-50	40-50	50-60			
	Ai	V 0.46	40-50	40-50?	50-60?	50-60?	60-70?		IV-30	17.5, 16
		IV 0.40	40-50	40-50	80-90	90-120				
		V 0.70	10-15	10-15	15-25	20-30	25-35	35-40?		
NH ₄ Cl	Ai	IV 0.40	20-30	20-30	30-40	30-40			V-15	16, 15.5
		1.00	5-15	5-15	20-30	120?	120?	130?		

Foot-note: 1) Ai *Asplenium incisum* Thunberg, Tj *Thelypteris japonica* Ching, Lt *Leptogramma totta* J. Smith Lm *Leptoromohra Miqueliana* H. Ito. 2) In the column of Remarks, the date, the room- and water-temperature in the experiment were represented respectively. 3) II, III, IV, V, and VI in the column of Mol. indicate the region in which these mol.-values are the limit concentrations. ? no clear observation.

closely to the transversal membrane which is assumed to be formed newly, in other words, this membrane may possess the high viscosity which disturbs the cell polarity. At the tangential or the radial polarity, in general, the prothallial cell

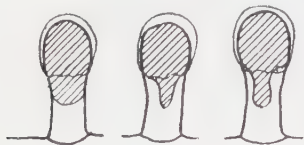


Fig. 4. The forms of plasmolysis of the glandular hair in *Thelypteris japonica* Ching. ($\times 240$). In 0.34 mol. glucose-solution. The portion of the oblique lines is that of the cytoplasm

begin the plasmolysis earlier at the apical pole than at the basal one, too, but occasionally this phenomenon is not clear. The cytoplasm of the glandular hair is observed to show, on the whole, the positive plasmolysis at the basal portion and the negative at the apical (i. e. 0.3, 0.6, 0.54 mol. urea, 0.3 mol. CaCl_2 , 0.22, 0.24 mol. KCl, 0.28 mol. KNO_3 , 0.34, 0.60, 0.68 mol. glucose) (Fig. 4). In HCl or ethyl alcohol solution the plasmolysis was negative.

II The Duration of Plasmolysis

At the portion of Region VI the form of plasmolysis of the prothallial cell could not be recognized clearly and no perfect form of A-type was found in general at the neighbourhood of Region V, whereas in the other regions the clear A-types could be observed especially in saccharose-, urea-, and KNO_3 -solutions. The duration of plasmolysis (plasmolysis time) is the one in which the form of plasmolysis gets to A-type since the incipient plasmolysis occurred, and it is decided as shown in Table II in which the extracts of the experiments were given.

The duration of plasmolysis is shorter at the basal pole than at the portion of the apical pole in the longitudinal polarity within the whole field of the prothallium and is shortest at Region I, and it becomes long gradually according as the region approaches Region VI. At the tangential or the radial polarity, though no exact observations were often got, the basal pole seemed to indicate also the short duration, compared with that of the apical pole. The more the solution of the plasmolyticum is hypertonic, the shorter the duration grows at each region. The difference of the species of the fern-prothallium or the plasmolyticum gives rise to that of the duration of plasmolysis more or less.

(to be continued)

Explanation of Plates

The polar plasmolysis of the prothallial cell in *Asplenium incisum* Thunberg.

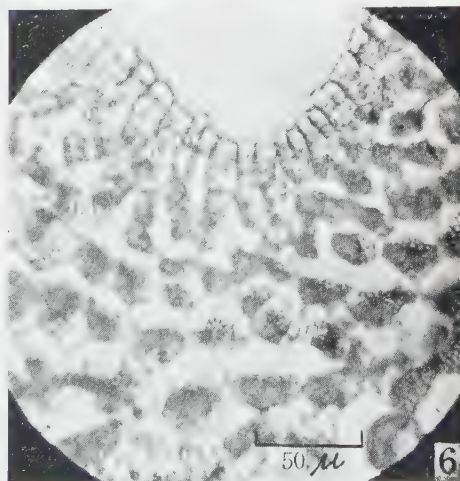
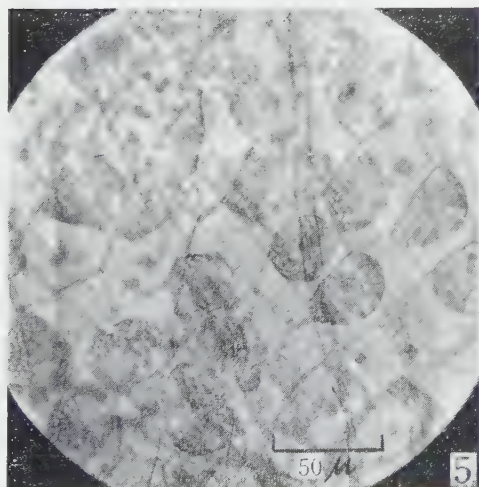
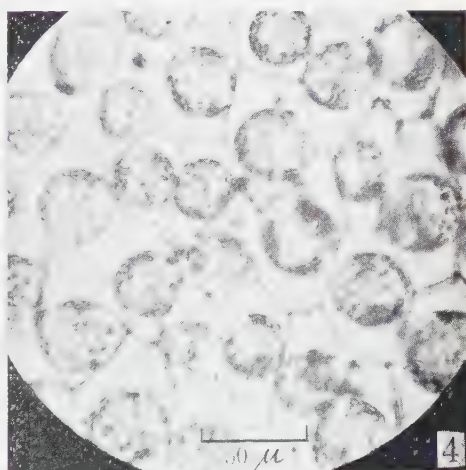
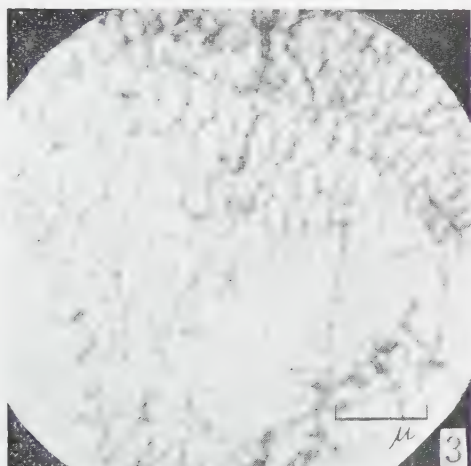
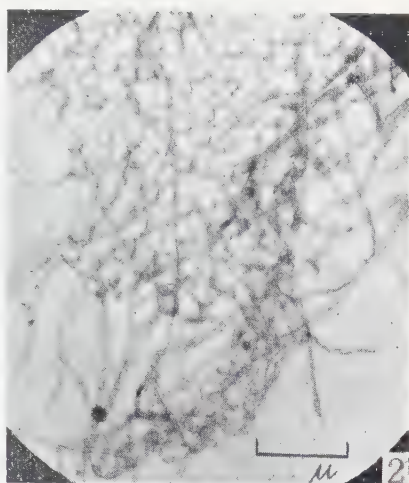
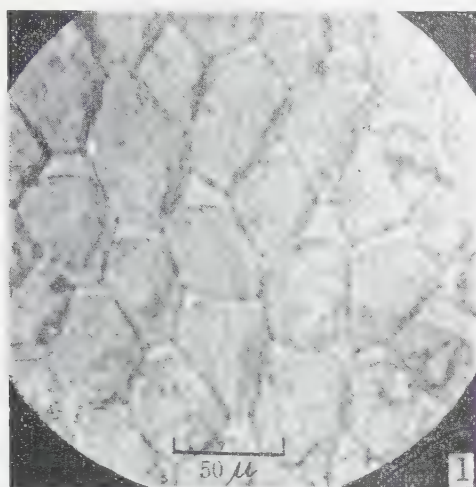
Photo. 1. Incipient plasmolysis in 0.36 mol. glucose-solution after four minutes. Region II.

Photos. 2, 3. Plasmolysis in 0.36 mol. MgCl_2 -solution after seven minutes. 2. Going to reach A-type in Region I and II. 3. The plasmolysis in Region III occurred slightly while in Region I and II are going to A-type.

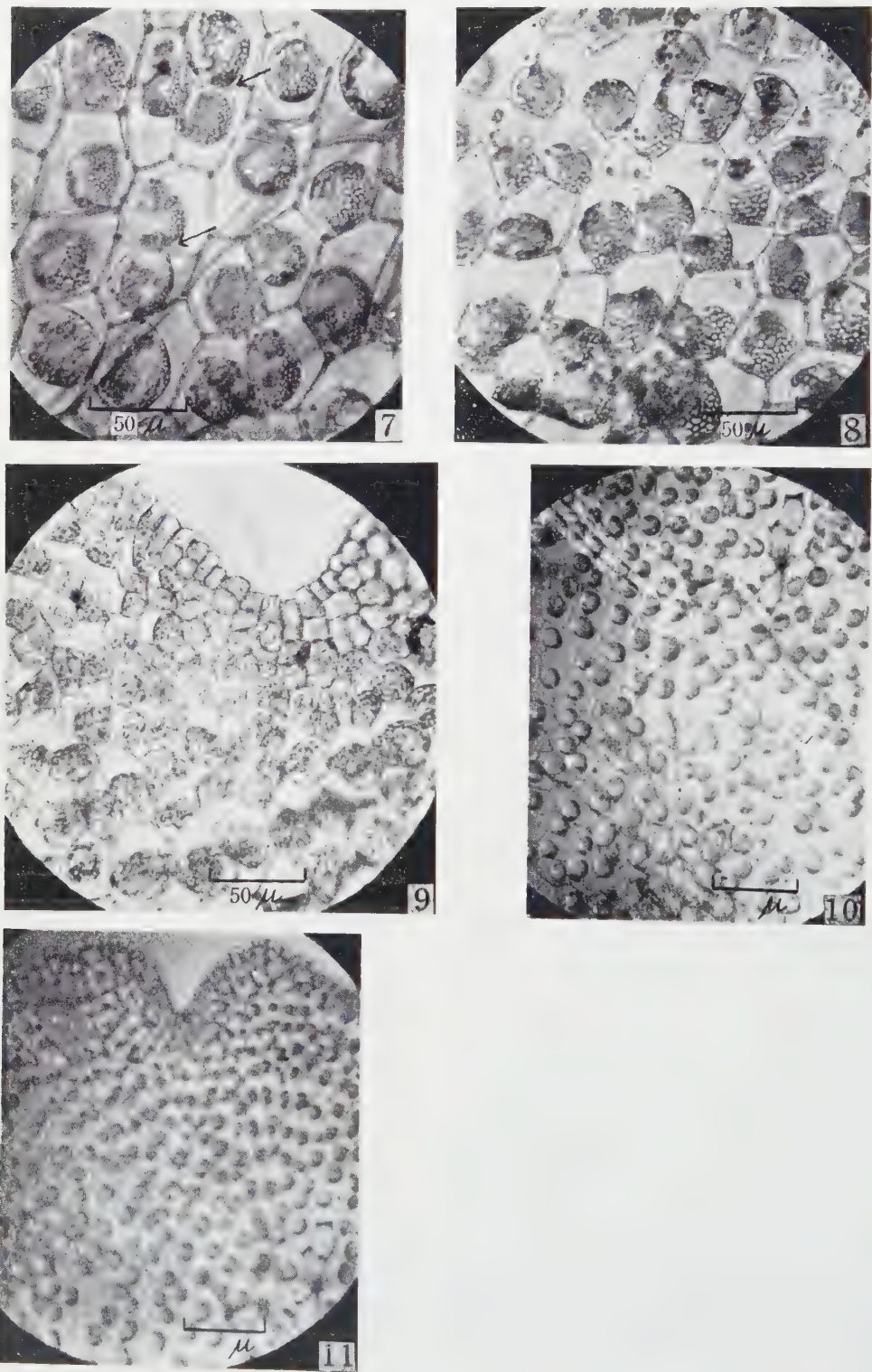
Photos. 4, 5, 6. Plasmolysis in 0.42 mol. KCl-solution after eight minutes. 4. The Region II; almost perfect A-type. 5. Region IV; advanced B-type. 6. Region V, VI; B- or C-type.

Photos. 7, 8, 9. Plasmolysis in 0.6 mol. saccharose-solution after fifty minutes. 7. Region III; almost A-type. 8. Region IV; advanced B-type. Not perfect A-type. 9. Region V, VI; B-type.

Photos. 10, 11. A-type forms of plasmolysis in 0.70 mol. urea-solution after seventy minutes. 10. Neighbourhood of Region II, III, and IV. 11. Neighbourhood of Region IV, V, VI.



I. Igura: Gametophytes of Ferns IX.



I. Igura: Gametophytes of Ferns IX.

キンボウゲ科の細胞学的研究 II

イチリンソウ属及びユキワリソウ属の核型

栗 田 正 秀*

Masahide KURITA: Cytological Studies in Ranunculaceae II

The Karyotypes of *Anemone* and *Hepatica*

1955 年 2 月 22 日受付

イチリンソウ属 (*Anemone*) の染色体はすでに多数の研究者たとえば高嶺¹⁶⁾, Langlet^{4, 5, 6)} Moffett⁹⁾, 松浦及須藤⁷⁾, Rosenthal¹³⁾, 水野⁸⁾, Böcher²⁾, Bernström¹⁾ 等によつて研究され、染色体基本数として 7 及び 8 が報告されて、Moffett, 松浦及須藤, Rosenthal 及び水野は本属の核型を研究した。イチリンソウ属に近縁なユキワリソウ属 (*Hepatica*) の染色体研究としては Langlet^{4, 5)}, 杉浦¹⁵⁾, Moffett⁹⁾, 松浦及須藤⁷⁾ 等の報告があり、その基本数は 7 とされている。Moffett 及び松浦及須藤は本属の核型についてのべている。しかしイチリンソウ属内の基本数 7 と 8 との関係及びこれらとユキワリソウ属の基本数 7 との関係を核型上から論議した報告はすくなく、ただ Moffett⁹⁾ がスハマソウ *Hepatica triloba* (= *Anemone Hepatica*) ($2n=14$) と基本数 8 の系列に属するイチリンソウ属植物との間の関係は染色体の合着によつて説明されると簡単にのべた報告があるだけのものである。

筆者も両属植物の核型を詳細に分析し、基本数 7 と 8 との関係を明らかにしたい目的で研究を行っているが、ここにその結果の一部を報告する。

方法は筆者の前報告におけると同様である

観 察

1. シュウメイギク *Anemone hupehensis* Lemoine var. *japonica* (Thunb.) Bowles et Stearn (愛媛県皿ヶ嶺産), サンリンソウ *A. stolonifera* Maxim. (栃木県日光産), ヤエザキサンリンソウ *A. stolonifera* Ma-

xim. var. *plena* Honda (長野県岩管山産), *A. virginiana* L. (北アメリカ産)

シュウメイギクの染色体は Moffett⁹⁾, 松浦及須藤⁷⁾ 等により, *A. virginiana* のそれは Dahl³⁾ によりすでに研究されており、いずれの植物も $2n=16$ と報告されている。

筆者もシュウメイギクの根端細胞で 16 個の染色体をかぞえた。これらは第 1 図に示すように構成員のそれぞれよく一致する 2 つの半数染色体組に区別できる。各組に属する 8 染色体のうち 5 個 (第 1 図, a-e) は V 字形, 残り 3 個 (同, f-h) は J 字形である。V 字形染色体は順次きわめてわずかに長さに差がみられ, 4 個 (同, a-d) はそれぞれ中部着糸点をもっているが, 他の 1 個 (同, e) の着糸点は次中部で前記 4 染色体にくらべて明らかに一端によつたところにあり, 短腕は長腕の $1/2$ よりもやや長い。J 字形染色体のうち 1 個 (同, f) は染色体の幅より長い短腕を, 1 個 (同, g) は染色体の幅より短い乳頭状の短腕をもっており, 最後の 1 個 (同, h) の短腕も小さい乳頭状であつて, その先端に付随体がある。サンリンソウ, ヤエザキサンリンソウ及び *A. virginiana* の各核型はシュウメイギクのそれとよく似ていて明らかな相違はみいだしえなかつた。したがつて前記 2 種 2 変種の核型は次の式で示せる。

$$K(2n)=16=8A^m+2B^{sm}+2C^{st}+2D_1^{st}+2^tD_2^{st}$$

Moffett は $2n=16$ をもつ種の核型を次のように決定した。すなわち 4 対は中部又は次中部着糸点をもち, 他の 1 対ではその短腕は長腕の約 $1/3$ であり, 残りの 3 対では着糸点は次端部にあつて, このうち 1 対ではその短腕に付随体があると報告した。筆者の結果は既述のごとくで V 字形染

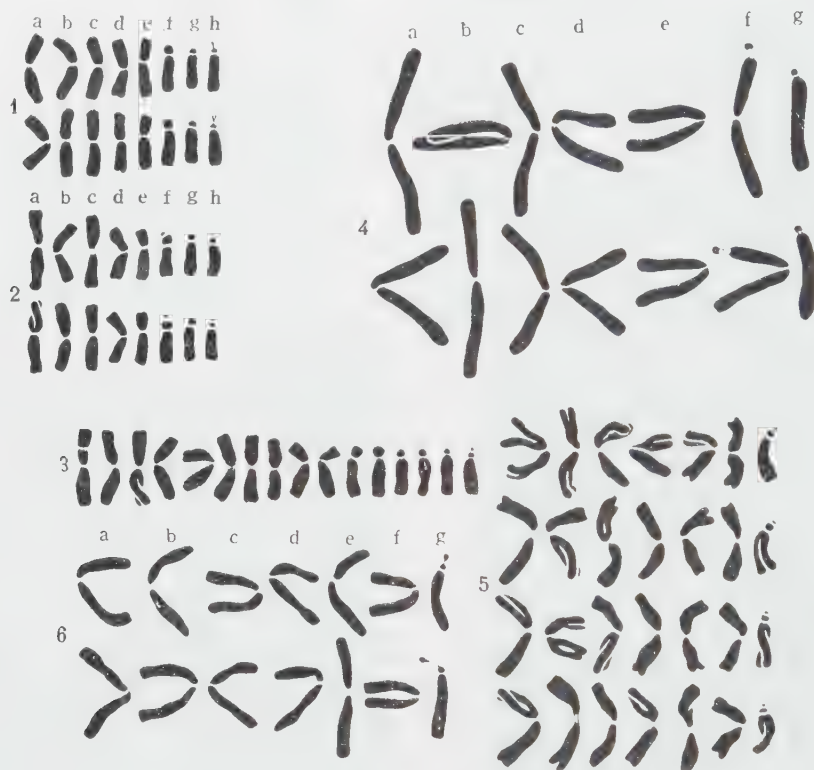
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色体についても Moffett のそれと多少ことなっており、3 対の J 字形染色体についても筆者はこれを短腕の大きさによつて 2 群にわけたが Moffett はこの点については何等言及していない。

2 イチリンソウ *A. nikoensis* Maxim. (愛媛

され $2n=16$ の染色体数が報告されている。

核型はイチリンソウのそれによく似るが次の点でことなる。すなわちイチリンソウでは付随体は J 字形染色体のうち大きい短腕をもつた 1 対と小さい短腕をもつた 1 対とにあるが、ペニバナオキ



Figs. 1-6. Somatic chromosomes. $\times 1110$.

- 1, *Anemone hupehensis* var. *japonica* 2, *A. nikoensis* 3, *A. hybrida*
4, *A. narcissiflora* 5, *A. Keiskeana* 6, *Hepatica acuta*

県上浮穴郡産)

イチリンソウの核型はシュウメイギクのそれと非常によく似ているが、ただ次の点でことなる(第2図)。すなわちイチリンソウでは J 字形染色体のうちで最も大きい短腕を有する染色体(同 f) もその短腕に付随体をもっており、したがって 2 対の付随体染色体が存在することである。

本種の核型は次のようにしめせる。

$$K(2n)=16=8A^m+2B^{sm}+2^tC^{st}+2D_1^{st}+2^tD_2^{st}$$

3. ペニバナオキナグサ *A. coronaria* L. (松山市内栽培)

本種の染色体は中島¹⁰⁾、水野⁸⁾等によつて研究

ナグサでは J 字形染色体のうちの小さい短腕をもつた 2 対にそれがみつめられることである。

したがって本種の核型は次の式で示せる。

$$K(2n)=16=8A^m+2B^{sm}+2C^{st}+4^tD^{st}$$

4. ボタンキブネギク *A. hybrida* Godron (松山市内栽培)

本種の核型は次の 2 点をのぞけば既述のシュウメイギクのそれとよく一致する(第3図)。すなわち 1 つはボタンキブネギクの V 字形染色体中の大形の 1 個(第3図、左端)はその 1 腕において中央よりやや内端よりに二次くびれをもっていることであり、他は体細胞にて付随体染色体が 1 個

(同、右端)しか存在しないことである。この付随体染色体数についてはすでに水野¹⁾が指摘したところであつて筆者の観察結果もこの点で同氏のそれと一致する。核型は次のように示せる。

$$K(2n) = 16 = 7A_1^m + {}^{cs}A_2^m + 2B^{sm} + 2C^{st} + 3D_1^{st} + {}^tD_2^{ts}$$

5. ハクサンイチゲ *A. narcissiflora* L. (栃木県日光産)

酒井¹⁴⁾及び Langlet⁶⁾によつて本種の染色体数は $2n=14$ と報告されている。

筆者も根端細胞で 14 個の染色体をみとめた。これらは第 4 図に示すように構成員のそれぞれよく一致する 2 つの半数染色体組に区分できる。各組の 7 染色体のうち 5 個 (第 4 図, a-e) はいづ

組の 7 染色体のうち 6 個は中部着糸点をもち順次わずかに長さに差がみとめられる。残り 1 個は次端部着糸でその短腕は小さく、かつ微小な付随体をもっている。

本種の染色体も $2n=16$ を有する種のそれよりやや大きい。核型は次の式で示せる。

$$K(2n) = 28 = 24A^m + 4{}^tB^{st}$$

7. ミスミソウ *Hepatica acuta* Britton (埼玉県武甲山産)

本種の染色体数は杉浦¹⁵⁾によつて $2n=16$, 中島¹¹⁾によつて $2n=14$ と報告された。筆者も中島と同様、根端細胞で 14 個の染色体をみとめたが、これらは構成員のそれぞれ一致する 2 つの半数染色体組に区別できる (第 6 図)。各組の 7 染色体のうち 6 個 (同, a-f) は中部着糸点をもち順

Table 1. Lengths of the chromosomes of *Anemone* and *Hepatica* in micron

Plant	Chromosome number (2n)	The largest chromosome	Ratio	The smallest chromosome	Ratio
<i>Anemone hupehensis</i> var. <i>japonica</i>	16	7.8	100	4.8	100
<i>A. stolonifera</i>	16	9.3	119	5.6	117
<i>A. stolonifera</i> var. <i>plena</i>	16	8.6	110	5.4	113
<i>A. virginiana</i>	16	8.0	103	5.0	104
<i>A. nikoensis</i>	16	7.9	101	4.6	96
<i>A. coronaria</i>	16	9.0	115	5.7	119
<i>A. hybrida</i>	16	8.8	113	4.8	100
<i>A. narcissiflora</i>	14	15.4	198	9.0	188
<i>A. Keiskeana</i>	28	11.9	153	6.9	144
<i>Hepatica acuta</i>	14	14.3	183	8.6	179

れも中部着糸点をもち順次きわめてわずかに長さに差がみとめられる。1 個 (同, f) は中部に近い次中部に着糸点をもち、短腕にはやや大きい付随体がある。残り 1 個 (同, g) は次端部着糸で短腕は小さく乳頭状となつている。

第 1 表に示すように本種の染色体は既述の各種の染色体よりかなり大きい。

核型は次のように示せる。

$$K(2n) = 14 = 10A^m + 2{}^tB^{sm} + 2C^{st}$$

6. ユキワリイチゲ *A. Keiskeana* T. Ito (愛媛県井内峠産)

本種の根端細胞で 28 個の染色体をみとめた。これらはその形態から第 5 図に示すように構成員のそれぞれ相似る 4 組に分けることができる。各

次わずかに長さに差がみられる。他の 1 個 (同, g) は次端部着糸で、その短腕は染色体の幅よりわずかに短く、かつ付随体をもっている。

本種の染色体もイチリンソウ属における $2n=16$ 染色体を有する種のそれよりかなり大きい。

核型は次の式で示せる。

$$K(2n) = 14 = 12A^m + 2{}^tB^{st}$$

その核型は Moffett⁹⁾, 松浦及須藤⁷⁾によつて研究された同属他種のそれとほぼ一致する。

考 察

イチリンソウ属 7 種 2 変種のうちハクサンイチゲとユキワリイチゲは基本数 7 の系列に、他は 8 の系列に属する。いま染色体の大きさ及び付随体

を考慮せずに、両系列の基本染色体組を比較すると最も顕著な差異は7系列のそれには8系列のそれよりJ字形染色体が2個少ないが、V字形染色体は1個多い。ユキワリソウ属ミスミソウの半数染色体組とイチリンソウ属内の8系列の種の基本染色体組との間にも同様に2Jと1Vの関係がみられる。すでに述べたようにMoffettも前記両属を研究し、2Jと1Vの関係を染色体合着によるとしたが、そのような単純な説では説明されえないであろう。染色体数の変化に結果する2Jと1Vとの関係については相当多くの動植物において論ぜられている(Navashin)が、本報告における両属植物の場合いかに説明するか、この点についてはさらに今後の研究にまきたい。

第1表に示すようにイチリンソウ属内で基本数7の系列に属するハクサンイチゲとユキワリイチゲの染色体は8の系列に属する種のそれより大き

い。この染色体の大きさの相違は両系列がわかれて以来生じたものであろうか。

ミスミソウの半数染色体組はハクサンイチゲの半数染色体組、特にユキワリイチゲの基本染色体組に似ており、いずれも他種よりその染色体が大きい。そしてイチリンソウ属内でユキワリイチゲ及びハクサンイチゲは同属他種とは相当ちがつた核型を示している。したがってミスミソウをイチリンソウ属からわけてユキワリソウ属として取扱うならば、核型上の観点だけからいつてユキワリイチゲもハクサンイチゲもミスミソウと同様に取扱うのが妥当ではなからうか。

御懇切な御指導をたまわつた下斗米教授に厚く御礼申し上げるとともに、材料の入手にあたり多大の御援助をいただいた久保田秀夫氏に深く感謝する。

Summary

1. Karyotype studies were made on eight species and two varieties in Ranunculaceae. The results obtained are as follows:

<i>Anemone hupehensis</i> var. <i>japonica</i>	}	$K(2n)=16=8A^m+2B^{sm}+2C^{st}+2D_1^{st}+2^tD_2^{st}$
<i>A. stolonifera</i>		
<i>A. stolonifera</i> var. <i>plena</i>		
<i>A. virginiana</i>		
<i>A. nikoensis</i>		$K(2n)=16=8A^m+2B^{sm}+2^tC^{st}+2^tD_1^{st}+2^tD_2^{st}$
<i>A. coronaria</i>		$K(2n)=16=8A^m+2B^{sm}+2C^{st}+4^tD^{st}$
<i>A. hybrida</i>		$K(2n)=16=7A_1^m+c^sA_2^m+2B^{sm}+2C^{st}+3D_1^{st}+^tD_3^{st}$
<i>A. narcissiflora</i>		$K(2n)=14=10A^m+2^tB^{sm}+2C^{st}$
<i>A. Keiskeana</i>		$K(2n)=28=24A^m+4^tB^{st}$
<i>Hepatica acuta</i>		$K(2n)=14=12A^m+2^tB^{st}$

2. From the karyotype analysis, the two *Anemone*-species, *A. narcissiflora* and *A. Keiskeana*, are more similar to *Hepatica acuta* than to the other *Anemone*-species.

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アルカリ土金属の蓆酸塩の晶癖とその植物学的意義

高 見 亘*

Wataru TAKAMI**: Crystal Habits of Oxalates of Alkaline Earth Metals
and Their Botanical Meanings

1955 年 3 月 28 日受付

蓆酸カルシウムの結晶の形や分布については古くから十分研究されており、^{1) Scott⁵⁾} は *Ricinus* で組織の分化に関連して考察し、Heintzelmann & Howard²⁾, Ueno⁷⁾ は分類学的立場から観察し魚の場合にも研究されている。^{3, 4)} しかし、何故に *Dioscorea* では針状結晶ばかりが現われるかについては十分な解答は得られていないので、筆者⁶⁾ も二三の報告をしたが、ここでは上の問題の一つの解答として、存在イオンの影響について述べる。植物体内の蓆酸カルシウムのこの見地からの研究は未だなされていないようで、化学的にも結晶の生長に対するイオンの影響に対しては多くの研究⁸⁾ があるが、蓆酸カルシウムのような結晶性沈澱の場合には知られていない。

材料及び方法

アルカリ土金属の塩と蓆酸を等発皿で混合させて蓆酸塩を作った。温度の影響については察してあるので⁶⁾、とくに指示していない場合には気温 9°C 内外で試みた。また、植物細胞中のアンモニウムイオンの検出にはネスレル指薬を用いて変色の程度で比較した。

実 験

(1) 標準の結晶形の決定

蓆酸カルシウムの結晶形は確定的なものではなく状況によつていろいろな形ができて、不安定である。例えば、ともに 0.5 モルの硝酸カルシウム 2 cc に蓆酸 2 cc を加えると、八面体のものまたはそれに新しい面が追加されたものが最も多くできるが、同じ状態で試みてもどちらが多くで

きるかはまちまちである。

実験の結果及び植物細胞内に見られるものから蓆酸カルシウムでは、正方晶系の八面体のもの (Fig. 1)、側面が梯形である六角形板状のもの (Fig. 2) 直方体のもの (Fig. 3) が標準形であると考えられる。そして、三軸の長さの比を測定して 1:2:2 であることが知られる。

それに対し、蓆酸バリウムや蓆酸ストロンチウムのはより安定で、前者のはずつと大きい柱形 (Fig. 4) で、後者のは、硝酸ストロンチウムと蓆酸とでは Fig. 5 のだけが、塩化ストロンチウムと蓆酸とでは Fig. 6 のように蓆酸カルシウムと同様なものが得られた。

(2) pH の影響

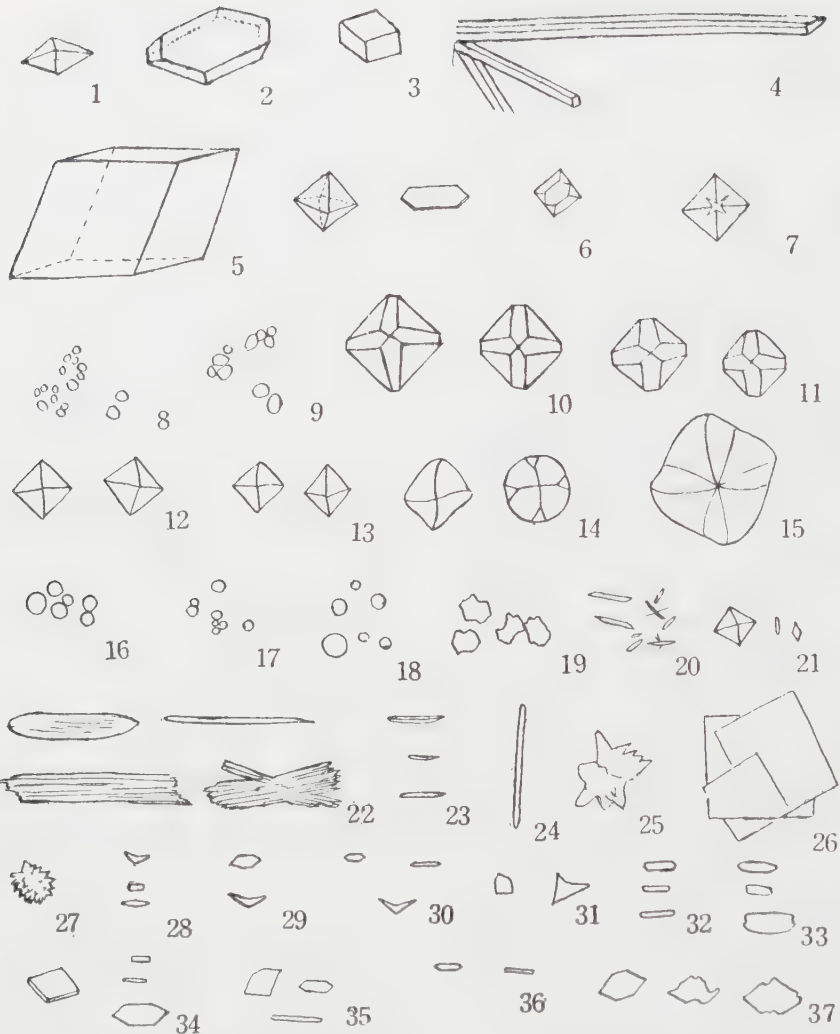
蓆酸カルシウムは塩酸、硝酸の稀薄溶液にとけるから pH の影響があることは明かで、pH を 7.8 にすると反応速度が大となり、Fig. 7 のような高温の場合に得られたような結晶⁶⁾ が得られた。次に、0.5 モル硝酸カルシウム 2cc (pH 5.6) を硝酸で pH 2.6 にした後、0.5 モル蓆酸 2cc を加えて pH 0.6 にしても Fig. 33 のようなものが混入したり、形が小さくなる以外に結晶形の変化は認められなかつた。したがつて、植物細胞の場合の pH の結晶形に対する影響⁹⁾ は断定的なものではないと考えられる。

(3) 陰イオンの影響

気温 9°C で、両液の濃度がともに 1 モルの場合に、硝酸カルシウムの方が、塩化カルシウムの場合よりも沈澱粒子は小さかつた (Figs. 8-9)。次に、気温 12°C で、両液の濃度がともに 0.5 モルと 0.1 モルの場合に得られた大形の八面体の結晶を比べると Figs. 10-13 のように、硝酸カルシウムの方が塩化カルシウムの場合より大きく、 NO_3^- より Cl^- の方が妨害作用が

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1~3. 蔞酸カルシウム標準の結晶×1000。4. 蔞酸バリウムの結晶×330。5. 蔞酸と硝酸ストロンチウムによる結晶。6. 蔞酸と塩化ストロンチウムによる結晶。7. pH=7.8のときの蔞酸カルシウムの結晶。8. とともに1モルの硝酸カルシウムと蔞酸による沈澱粒子。9. 同じく1モル塩化カルシウムによる沈澱粒子。10. とともに0.5モルの硝酸カルシウムと蔞酸による結晶。11. 同じく塩化カルシウムによる結晶。12~13. 10~11のに対し0.1モルの場合。14. SO_4^{--} による歪形×600。15. 同じく SO_4^{--} による歪形×1200。16-18. Cu^{++} による蔞酸カルシウム、蔞酸バリウム、蔞酸ストロンチウムの結晶。19-20. Mg^{++} による蔞酸カルシウムの結晶。21~23. NH_4^+ による蔞酸カルシウムの結晶。25. モミジの葉の蔞酸カルシウムの結晶。25~26. 二種のペゴニアの葉の結晶。27-31. それぞれオオマツヨイグサ、チャ、ムクゲ、サルスベリ、ユリの葯の結晶。32-33. スイセンの葉に作らせた結晶。34~87. クノツプ液にそれぞれ KNO_3 , $(\text{NH}_4)_2\text{HPO}_4$, NH_4OH , MgO を加えた場合のミヨウガの葉の結晶×600。

強いと推定される。さらに、これらの双方の場合より SO_4^{--} , OH^- の順に結晶形は小さくなると判断され、硝酸カルシウムに塩化カルシウムを混

じた場合には両者の中間の大きさになった。また、硝酸カルシウムまたは塩化カルシウムに硫酸カルシウムを混じておくと、軸がねじれたり、周

辺がふくらんだりして形が不整なものが見られたことは興味深い (Figs. 14-15)。

(4) 陽イオンの影響

H⁺ 以外の陽イオンの影響を見るのに、すべて 0.1 モルのものを使つて、硝酸カルシウムに少量の硝酸銅を、塩化バリウムに少量の塩化銅を、塩化ストロンチウムに少量の塩化銅を混じたものに硝酸を加えるとどれも不定形粒子になつた (Figs. 16~18)。これらの場合に pH の影響は考慮にいれる必要のないことは、例えば最初の場合には、硝酸を加えた最後の pH は 3.2 で、硝酸カルシウムと硝酸だけの場合の pH に等しい。次に、塩化カルシウムまたは塩化ストロンチウムに塩化鉄を混じた場合には結晶は全く見られないが、この場合には pH がずつと小さくなるので Fe⁺⁺ と H⁺ との両方の影響であると考えられる。その他 Na⁺, K⁺, Mg⁺⁺, NH₄⁺ の影響をも観察した。Na⁺, K⁺ の場合には、硝酸カルシウムに硝酸ナトリウムまたは硝酸カリウムを混じておくと結晶形はやや小さくなり、硫酸カルシウ

に結晶は見られなかつた。Mg⁺⁺ の場合にはそれ程でもなく、すべて 0.1 モルのものを使うとき、塩化物に塩化マグネシウムを混じて観察すると、不定形化や針状化が見られた (Figs. 19-20)。NH₄⁺ の場合も同様で、すべて 0.5 モルの塩化カルシウム、塩化アンモニウムと硝酸とですでに形が小さくなつたり、針状形が現われた (Fig. 21)。さらに、NH₄⁺ の影響を強くして、0.5 モル塩化アンモニウム 4cc と 0.1 モル塩化カルシウム 1cc の混合液に 0.1 モル硝酸を加えると反応速度は小となり、針状化はさらに促進された。

Figs. 22-23 はそれぞれ硝酸アンモニウムと硝酸カルシウムとともに 0.5 モル及び 0.1 モルで反応させた場合の結晶形で、植物細胞内に見られる針状のものや針状束を示した。

(5) 植物細胞の NH₄⁺ の硝酸カルシウムの結晶形に対する影響

植物細胞内の NH₄⁺ の多少をネスレル指薬で比色によつて比較しても大に変異があることが知られる。(表 1-3) 一般に硝酸カルシウムの針状

表 1. 稚葉における NH₄⁺ の比較

イ	チ	ゴ	++++	キ	ク	++	ヤ	マ	ノ	イ	モ	++	ム	ク	ゲ	+
ガ	ジ	イ	チ	ゴ	++++	オ	オ	バ	コ	++	コ	ス	モ	ス	++	レ
カ		シ	++++	ス	イ	セ	ン	++	ム	ラ	サ	キ	ツ	ユ	ク	サ
モ	ミ	ジ	++++	ビ	ョ	ウ	ヤ	ナ	ギ	++	チ		ヤ	++	タ	マ
サル	ス	ベ	リ	++++	ム		ベ	++	コ	ム	ギ	+			フ	ジ
マ		ツ	++	ヤ	ツ	デ	++	ユ		リ	+		ヤ	ブ	ラ	ン
ア	オ	キ	++	カ		キ	++	ユ	ズ	リ	ハ	+	ア	オ	ギ	リ

表 2. 葉柄における NH₄⁺ の比較

サ	ザ	ン	カ	+++	レ	ツ	クス	・	ベ	ゴ	ニア	+	ヤ	ツ	デ	-
ユ		リ	++	ベ	ゴ	ニ	ア	-	オ	オ	マ	ツ	ヨ	イ	グ	サ
ヤ	ブ	カ	ラ	シ	+	ア	オ	ギ	リ	-	キン	モ	ク	セ	イ	-

表 3. 莖における NH₄⁺ の比較

コ	ス	モ	ス	+++	ム	ラ	サ	キ	ツ	ユ	ク	サ	+
サル	ス	ベ	リ	+++	ヤ	ブ	ラ	ン	-				
ム	ク	ケ	+++	タ	マ	ス	ダ	レ	-				
チ	ヤ	+++	カ	ボ	チ	ヤ	-						

ムと硫酸ナトリウムの混合液の pH が 5.8 のものに、硝酸の等量を加えると pH は 1.4 となり、硝酸の半量を加えると pH は 2.2 となつてとも

結晶は単子葉植物の特徴であるとされているが、Fig. 24 に示すようにモミジの葉には針状に近いものが見られ、カキの葉にも棒状の結晶が見られ

た。また、ベゴニアの 2 種において、葉に NH_4^+ のあるものとないものがあり、前者では Fig. 25 のような結晶が、後者では八面体または Fig. 26 のような結晶が見られ、これは NH_4^+ の影響と考えられる。

次に、薔の中には NH_4^+ の濃度の大きいものが多く、コスモスとムラサキツユクサのは針状結晶で、その他の NH_4^+ のあるものでは Figs. 28-31 のように標準の形以外に凹四角形状のものが多数見られた。観察したもののうち NH_4^+ がなくて結晶を含むのはオオマツヨイクサであつた (Fig. 27)。

スイセンの葉はコムギの葉に比べて NH_4^+ が多く、コムギには殆ど結晶が見られないが、スイセンには針状結晶が多い。原形質分離をした細胞を 0.1 モルの酢酸に浸して観察すると、コムギの場合には八面体のものばかりであつたが、スイセンでは小形の針状のものや直方体のものも見られ、原形質分離によつて浸出した細胞液中には Fig. 32 のようなものが見られた。また、スイセンを 0.1 モルの硝酸カルシウムだけで水耕したものの表皮を原形質分離させた後、0.1 モルの酢酸に浸して観察すると結晶砂のほか Fig. 33 のような形の結晶が見られたが、これは NH_4^+ の影響によるものと考えられる。

(6) ミョウガの水耕

ミョウガの根茎をクノップ液 (KNO_3 1g, MgSO_4 1g, KH_2PO_4 1g, $\text{Ca}(\text{NO}_3)_2$ 3g を水 11 に溶かす) 及び $(\text{NH}_4)_2\text{HPO}_4$, MgO , Na_2SO_4 , KNO_3 各 1g 及びアンモニア水少量づつを加えた 6 種によつて水耕して稚葉の結晶を調べた。ミョウガの酢酸カルシウムの結晶は六角形板状のものが多く (Fig. 34), $(\text{NH}_4)_2\text{HPO}_4$ を加えたものでも NH_4^+ の反応は見られないが、その場合には Fig.

35 のように結晶形は五辺形のものや、細長いものが多かつた。アンモニア水を加えて葉に NH_4^+ の反応が見られた場合には大形のは稀で、Fig. 36 のようなものが多かつた。また、 MgO を加えた場合には、Fig. 37 のように形が不整なものも見られた。

結 論 及 び 論 議

本研究の結果によると、アルカリ土金属の酢酸塩の結晶形に対する pH の影響は、ある範囲では著しくないが、陰イオンの結晶形成に対する妨害作用は NO_3^- , Cl^- , SO_4^{--} , OH^- の順に強いことがわかる。また、 SO_4^{--} の歪状化する作用も注意すべきであろう。

次に、一般に、陰イオンよりも陽イオンの方が妨害作用が強いようで、とくに、 Mg^{++} 及び NH_4^+ による針状化の作用（これも妨害作用の一種である）は実際の植物細胞内で、何故針状形が現われるかの理由を解く鍵を与えるものと考えられる。針状形が現われる理由に滴加や粘性があげられるが⁶⁾、それら以上に介在イオンの影響の方が大きいと思われる。薔の中に現われる凹四角形状のものは前報⁶⁾ Fig. 10 のものの一部分が NH_4^+ の影響によつてこのような形になつたものと解される。

ムラサキツユクサの薔の NH_4^+ の濃度はあまり大きくないが、カルシウムまたは酢酸の濃度が小さいために影響が強くすべて針状結晶になると考えられる。それはスイセンの葉の実験で、0.1 モルの酢酸によつて八面体のものや針状のものが作られたことによつても推察される。ミョウガのような NH_4^+ の少ない葉を使つて NH_4^+ による針状化や、 Mg^{++} による不整化を検することのできた。

Summary

In the present investigation, the effects of various ions on the crystal habits of oxalates of alkaline earth metals were observed.

(1) The standard form of the calcium oxalate has a ratio of 1:2:2 of three axial lengths.

(2) The effect of pH is not so strong, if the variation range of pH is small.

(3) Disturbing effect of NO_3^- , Cl^- , SO_4^{--} and OH^- increases according to this order and complex effect of NO_3^- and SO_4^{--} is remarkable.

(4) Disturbing effects of positive ions is generally stronger than that of negative ions. Effects of Cu^{++} , Fe^{++} , Na^+ , K^+ , Mg^{++} , NH_4^+ were observed. Especially in the cases of Mg^{++} and NH_4^+ , action of transforming things into needle-like shape "raphide" is observed and compared with the form in the plant cells.

(5) Existence and non-existence of NH_4^+ in the various cells were checked and the above experiments could be positively examined in this case, and the related experiments were done for this reason. As the results of above considerations, one may conclude for the first time that one and the chief reason for "raphide" formation, formerly considered as an attribute of the Monocotyledoneae is due to the existence of NH_4^+ .

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蘚類数種の染色体

IX ハイゴケ属の核型, 性染色体及び倍数性

矢 野 孝 二*

Koji YANO*: On the Chromosomes in Some Mosses

IX. Karyotype, Sex Chromosome and Polyploidy of Genus *Hypnum*.

1955 年 3 月 19 日受付

筆者はさきにハイゴケ属 (*Hypnum*) の 2 種 *H. plumaeforme* Wils., *H. Fujiyamae* (Broth.) Par. の染色体数を $n=10$ と報告した(矢野1952)。今回同属の他種でこれと異なる染色体数 $n=6$ 及び $n=11$ のものを見出したので, これ等各種の核型及びそれ等の異質染色体の観察を行い, 尚そのうちの 2 種では性染色体を見出すことが出来た。以下これ等の結果を報告する。

研究に用いられた蘚の種名並びに採集地は次の如くである。固定並びに染色法は前報告の場合と同様である。

種 名	採集地
<i>Hypnum circinatum</i> Schimp.	越後：雨飾山, 妙高山
<i>H. plumaeforme</i> Wils.	越後：高田市
<i>H. Fujiyamae</i> (Broth.) Par.	信濃：五地藏岳
<i>H. reptile</i> Michx.	信濃：八ヶ岳

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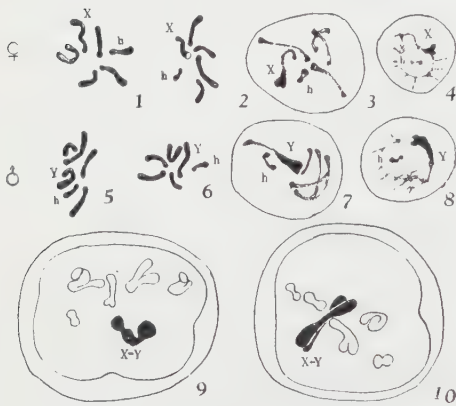
観 察

1) *Hypnum circinatum*

雌雄異株。染色体数は $n=6$ で、これは本属の基本数である (Figs. 1—10)。

雌株の核型は $K(n)=6=V(X)+2V+2J+m$ (h)。核板中最大の染色体 (V) は異質染色体 H であり、且これは性染色体 X である。X は一腕の端部に明瞭な二次狭窄を有する。この二次狭窄より外方の尾部及び他腕の大部分が異常凝縮を示す。最小の染色体 m は既報の各属の薺におけると同様に異質染色体 h であり、その異常凝縮は休止核では仁内の中央部に認められるので、いわゆる “Nukleolus-chromosomen” である。其他の常染色体は大小 2 個の V, 小形な 2 個の J より成る。

雄株の核型は $K(n)=6=V(Y)+2V+2J+m$ (h)。核板中最大の染色体 (V) は H である。こ



Figs. 1—10. *Hypnum circinatum* の染色体と異常凝縮 Chromosomes and heteropycnoses in *Hypnum circinatum*. 1—4. ♀ Gametophyte. 5—8. ♂ Gametophyte. 9, 10. Meiotic chromosomes in SMC's $\times 4000/3$

の形態は中期では X と同様で両者を区別することは困難であるが、前期又は休止核における異常凝縮の状態を比較すると両者の間に僅かな差異があるのでこれを Y とする。即ち Y も X と同様その異常凝縮部は二次狭窄を有しない方の腕の大部分及び他腕の端の小部分であるが、一般に Y の方が X より異常凝縮性が顕著である。即ち前期では Y の方が異常凝縮塊が幾分大きく、且染色

性も強い。休止核中ではこの差は一層明瞭で、Y に由来する染色質塊は X のそれに比して例外なく大きい。X, Y 以外の染色体は全く雌株、雄株共通であつて、両者の間に何等の相違が認められない。

本種では子嚢体における孢子母細胞内の異常凝縮及び減数分裂の観察も行つた (Figs. 9, 10)。この異常凝縮においては X と Y とが同一核内に存在する為両者の比較が容易であつた。即ちこの場合も常に Y の異常凝縮塊は X のそれに比して大きく且顕著であつて、両者の差は明瞭であつた。減数分裂の第一分裂中期には 6 II が認められ、そのうちの 1 個は他の 5 個に比して特に大きく、これは X—Y の対合である。

2) *Hypnum reptile*

雌雄同株。 $K(n)=11=2V(H)+4V+4+m(h)$ 。本種は $n=11$ であるから前記基数種に比すれば



Figs. 11—18. *Hypnum reptile* の染色体と異常凝縮 Chromosomes and heteropycnoses in *Hypnum reptile*. 11—14. Metaphase chromosomes and heteropycnoses in gametophytes. 15. Metaphase chromosomes in sporophyte. 17, 18. Meiotic chromosomes in SMC's $\times 4000/3$

低二倍種である。Figs. 11, 12 に示す如く本種の核板には 3 個の特に大きな染色体が認められる

が、このうちの 2 個は明瞭な異常凝縮を示すので H である (Figs. 13, 14)。この両 H の中期核板における大きさ、形態は等しく何れも V であり、且一腕の端部に二次狭窄を有する。然し両者の異常凝縮を比較すると互にやや異り、一方は他に比し異常凝縮塊が大きく、且染色性も強い。この差は休止核中では一層明瞭である。この両 H の形態及び異常凝縮性はそれぞれ前記基数種の性染色体 X 及び Y のそれによく似ている。このことは本種が雌雄同株であることと関連し興味ある事実である。尚本種には微小な m が 1 個認められるが、これは h である。他の 8 個の常染色体のうち 4 個は比較的大きな V で、特にこのうちの 1 個は前述の如く、その大きさ形態が H に匹敵するが他の 3 個はこれよりも小形である。他の 4 個は median 又は submedian の狭窄をもつ如くであるが、何れも小形な為これ等の形態は充分明らかにし得なかつた。

子囊体においては 22 個の染色体が見られる (Fig. 15)。胞子母細胞の減数第一分裂中期では 11 個の二価染色体が見られる (Figs. 16—18)。このうち 2 個はその大きさ、形態から 2 組の H-H の場合であることがわかる。これ等の二価染色体には不等対は認められず、後期には規則正しく分離して両極に向う。

3) *Hypnum plumaeforme*

雌雄異株。既報 (矢野 1952) の如く $n=10$ であるから本種も亦低二倍種である。今回本種の核型等を前記兩種と比較する為再観察を行つた。その結果本種でも前記 *circinatum* とほぼ同様な性染色体があることがわかつた (Figs. 19—28)。即ち本種の核型は $K(n)=10=V(X)+4V+4+m(h)$, $\delta K(n)=10=V(Y)+4V+4+m(h)$ である。即ち性染色体 X, Y は *circinatum* の場合と同様、雌雄何れにおいても最大の染色体である。そして中期においては両者の間に形及び大きさの差がなく、両者は異常凝縮においてのみ互に異り區別出来る。即ち休止核中では Y の異常凝縮は X のそれに比して大きく且明瞭である。他の染色体に関しては雌雄株間に何等の相違が認められない。即ち雌雄何れも最小の染色体 m は h であり、これは Nukleolinus-chromosomen である。又 8 個の常染色体のうち 1 個は H に次で大

きな V であり、これは一腕端に明瞭な二次狭窄をもっている。これに次で大きな 3 染色体も V である。其他の 4 染色体は小形であつて、これ等は median 又は submedian の狭窄をもつようであるが未だそれ等の形態を詳かになし得ない。尚これ等の常染色体の数並びに個々の形態は上記 reptile のそれ等によく似ている。

本種の子囊体では 20 個の染色体が認められ、又その異常凝縮では X と Y とが同一核内に共存する為両者の異常凝縮性の差を明瞭に認めることが出来た (Figs. 27, 28)。胞子母細胞の減数第一分裂中期には 10 II が見られる (Figs. 30, 31)。これ等には不等対は認められなく、後期には何れ



Figs. 19—31. *Hypnum plumaeforme* 及び *H. Fujiyamae* の染色体と異常凝縮 Chromosomes and heteropycnosis in *H. plumaeforme* and *H. Fujiyamae*. 19—28, 30, 31. *H. plumaeforme* 19—22. ♀ Gametophyte. 23—26. ♂ Gametophyte. 27, 28. Sporophyte. 30, 31. Meiotic chromosomes of the 1st metaphase and anaphase in SMC's. 29. *H. Fujiyamae*. $\times 4000/3$

も同大の染色体に分離して両極に向う。然しこれ等の二価染色体のうち最大の 1 個は、これを構成する 2 染色体がその一部分でのみ接合している為しばしば不規則な形態を示し、又この二価染色体は後期には他の染色体よりも遅れて両極に達する。これは X-Y の対合と推定される。

4) *Hypnum Fujiyamae*

雌雄異株。既報(矢野 1952)の如く $n=10$ であるから本種も上記 *plumaeforme* と同様な低二倍種である。今回前記の各種と比較の為本種の雌株につき再観察を行つた。核型は $K(n)=10=V(H)+4V+4+m(h)$ 。最大(V)及び最小(m)の染色体はそれぞれ H, h であり、これ等は上記の *plumaeforme* のそれ等によく似ている。然し常染色体の形態は *plumaeforme* 又は *reptile* のそれ等と僅かに相違している。即ち Fig. 29 に示す如く本種の中期核板には比較的大きな染色体が5個認められる。このうち1個はHであるが他の4個は何れもVであり、これ等のVの大きさは *plumaeforme* 等のこれに相当する染色体に比し幾分大形である。

考 察

ハイゴケ属(*Hypnum*)は多数の種を含んでいるが染色体の観察が行われたものは少く、僅かに Heitz (1928) によつて *H. imponens* $n=6\sim7$, 及び Vaarama (1950) によつて *H. cupressiforme* 10 II* の染色体数が報告されているに過ぎない。今回筆者によつて染色体の観察が行われた本属4種の雌雄性と核型は次の如くである。

Hypnum circinatum

$$\text{♀ } K(n)=6=V(X)+2V+2J+m(h)$$

$$\text{♂ } K(n)=6=V(Y)+2V+2J+m(h)$$

H. reptile

$$\text{♀ } K(n)=11=2V(H)+4V+4+m(h)$$

H. plumaeforme

$$\text{♀ } K(n)=10=V(X)+4V+4+m(h)$$

$$\text{♂ } K(n)=10=V(Y)+4V+4+m(h)$$

H. Fujiyamae

$$\text{♀ } K(n)=10=V(H)+4V+4+m(h)$$

これ等の染色体数は $n=6, 10, 11$ であつて正倍数性の関係を示していない。然し核型を比較すればそれ等は特異な倍数関係になつていことがわかる (Fig. 32)。即ち *circinatum* ($n=6$) を基数種として他の3種はこれの低二倍種に相当する。即ち *reptile* ($n=11$) は二倍体から h が1個不足するもの、*plumaeforme* ($n=10$) 及び

* Delay (1953) による。

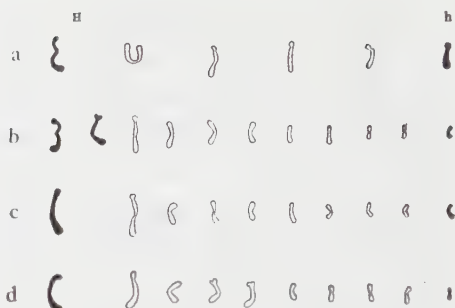


Fig. 32. *Hypnum* 属4種の配偶体の染色体 Serial alignments of gamete chromosomes of four species examined.

a. *Hypnum circinatum* (from Fig. 1).

b. *H. reptile* (from Fig. 12).

c. *H. plumaeforme* (from Fig. 19).

d. *H. Fujiyamae* (from Fig. 29).

Fujiyamae ($n=10$) は二倍体から H 及び h が各1個づつ不足する低二倍種と解釈出来る。この様に倍数体において異質染色体 H 又は h のみが不足している例(Heitz 1942, 矢野 1952, '54), 及び反対に異質染色体が過剰に加わっている例(辰野 1953, '41, 矢野 1954) は既に数種の蘚苔類で報告されており、そして筆者(1952)は此の現象は異質染色体の特異性に基因して生じたものであらうと推察した。今回再び同様な異質染色体の不足した低二倍種の例が見出されたことは、この様な倍数体の例が蘚苔類には可成多いことを示すものであり、これは異質染色体をもつた生物の倍数体における一特性として興味がある。

尙 Heitz (1928) が *H. imponens* の染色体の概数を $n=6\sim7$ と報告しているが、これはおそらく本属の基本種で $n=6$ であり、亦 Vaarama (1950) が *H. cupressiforme* で 10 II を報告しているがこれはおそらく筆者の *H. plumaeforme* 等と同様な低二倍種ではあるまいか。

circinatum では性染色体(X, Y)が見出された。その X 及び Y は染色体の形態では差がなく、異常凝縮性においてのみ差を示すものである。本種に比すれば *reptile* はその低二倍種であり、且雌雄同株であるが、その2個の H はその形及び異常凝縮性においてそれぞれ *circinatum* の X 及び Y と酷似している。よつて *reptile* の2個の H は *circinatum* の如き雌雄異株の基本種の性染色体 X 及び Y から導かれ、それ等を併有

することからこれが雌雄同株となつているものではあるまいか。従つて本種の核型は次の如く示さるべきものであろう。

$$K(n)=11=2V(X, Y)+4V+4+m(h)$$

次に *plumaeforme* は同じく低二倍種であるが、これは雌雄異株である。即ちこれは上記雌雄同株の *reptile* の如く 2 個の H を併有しないで 1 個の H を失つている。即ち雌株、雄株それぞれ 1 個宛の H を有し、それがそれぞれ X 及び Y であつた。それが爲に此の低二倍種は雌雄異株となつているものであろう。

Hypnum 属 4 種の基数種と各倍数種の常染色

体を比較すれば、前者は 4 個後者は 8 個で丁度 2 倍になつているが、倍数種は必ずしも基数種と同形態の染色体を二重にもつているのではなく、各倍数種はそれぞれ特色ある染色体組を示している。即ち各倍数種の 8 個の常染色体は相同形の、4 個づつの 2 組に分けることは困難である。従つてこれ等の低二倍種は異常のゲノムの合一によつて導かれた異質倍数体であるか、或は同質のゲノムの倍加によつて導かれた同質倍数体が長年月の間に染色体の形態の変化を起し異質化したものかの何れかであらう。

Résumé

1) The karyotypes of examined four species belonging to the genus *Hypnum* are as follows;

Hypnum circinatum Schimp.

$$\text{♀ } K(n)=6=V(X)+2V+2J+m(h)$$

$$\text{♂ } K(n)=6=V(Y)+2V+2J+m(h)$$

Hypnum plumaeforme Wils.

$$\text{♀ } K(n)=10=V(X)+4V+4+m(h)$$

$$\text{♂ } K(n)=10=V(Y)+4V+4+m(h)$$

Hypnum Fujiyamae (Broth.) Par.

$$\text{♀ } K(u)=10=V(H)+4V+4+m(h)$$

Hypnum reptile Michx.

$$\text{♀ } K(n)=11=2V(H)+4V+4+m(h)$$

2) *H. circinatum* is the basic species of this genus, and the other three are the hypodiploid species missing one h (*H. reptile*) or one set of H and h (*H. plumaeforme*, *H. Fujiyamae*).

3) The sex chromosomes X and Y which have been found in *H. circinatum* and *H. plumaeforme* are the same in chromosomal morphology, but there was observed a slight difference between their heteropycnosis.

4) The two H chromosomes of monoecious *H. reptile* may be the sex chromosomes X and Y, because their morphology and heteropycnosis are similar to those of the sex chromosomes X and Y of *H. circinatum*, etc.

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3. Tatuno, S. Bot. Mag. Tokyo 69: 892-898 (1935) 3. Jour. Sci. Hiroshima Univ. Ser. B. Div. 2, 4: 73-187 (1941)
4. Yano, K. Bot. Mag. Tokyo 65: 769-770 (1952); 67: 129-138 (1954)

本 会 記 事

昭和 29 会 計 年 度 決 算 報 告 (昭和 29 年 4 月 1 日から 30 年 3 月 31 日まで)

収 入 の 部			支 出 の 部			
会	費	599,640円	出	版	費	759,586円
バックナンバー売上金		97,743	発	送	費	160,360
別	刷	63,019	編	集	関 係 費	19,653
文 部 省 刊 行 補 助 費		230,000	図 書	関 係 費		46,445
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			支 部	補 助		10,000
			幹 事	手 当		79,000
			小	計		1,234,901
			次 年 度 繰 越 金			231,901
繰	計	1,466,802	繰	計		1,466,802

近 畿 支 部

支部総会(昭和 30 年 5 月 22 日(日), 於大阪大学・理学部)

1. 講演: 1) *Ceratium trichoceros* の核分裂(植田勝巳, 京大.理.植), 2) Agarography 及び塩析滴定法による生体構成蛋白 Component の検索(桃谷好英, 京大.理.植, 曾我美勝, 井上康夫, 京大.医.生理), 3) Picolin 滴定によるミトコンドリア構成成分の検索(曾我美勝, 井上康夫, 京大.医.生理, 桃谷好英, 京大.理.植), 4) アトモメーターからの蒸発速度を自記せしめる装置の試作(村田茂三, 京大.気象研), 5) 担子菌 *Psilocybe* の子実体形成に対するバクテリアの刺戟効果について(浦山隆司, 京大.農生), 6) エノキタケの子実体形成特に低温効果について(衣川堅二郎, 古川久彦, 京大.農生), 7) 竹筴科六属の実生の知見について(高木虎雄, 園部高校)

2. 支部長, 庶務, 会計幹事改選
3. 会計報告, その他

新 入 会

大野照好(九州) 鹿児島大教育生物・鹿児島市高麗町 684

小泉晴一(北海道) 釧路市富士見町 71 釧路湖陵高校・同校職員寮

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河野 清(近畿) 京都工芸繊維大・京都市右京区嵯峨一本木町 1

千葉勝朗(東北) 秋田市秋田県片林務部施業課・同市寺内将軍野四区原田喜久治方

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榊井 孝(関東) 横須賀市浦賀芝生 255 浦賀中学校

本会会員 志波清時氏は昭和 30 年 2 月 12 日死去されました。

ここに報告し謹んで哀悼の意を表します。

日 本 植 物 学 会

A Cytotaxonomic Comparison of Parsley and Celery

by Minosuke HIROE*

広江美之助： パースレーとセリリーの細胞分類学比較

Received April 9, 1955

In 1814, Hoffman established the genus *Petroselinum* based on *Apium Petroselinum* L. The principal reason advanced was that in *Apium* the petals are white, while in *Petroselinum* they are very light green. The writer believes that petal color is likely to be of very little taxonomic value.

In species of the genus *Apium*, especially *Apium Petroselinum* L., the lateral branches appear to be the axis, forming a monopodial sympodium, while the true stem apex produces an inflorescence. In many Umbelliferae, on the other hand, growth is by quite ordinary monopodial branching. The fruit of the genus *Apium* is small and ovoid or orbicular. Both *Apium graveolens* (celery) and *A. Petroselinum* (parsley) contain the glucoside characteristic of *Apium*.

From a study of *Apium Petroselinum*, Ogawa¹⁾ concluded that the chromosome number is $n=11$. Recently, Warscher²⁾ reported *Apium graveolens* to possess the chromosome number of $n=11$, also. In the present cytological observations on the root-tip cells of *A. Petroselinum* and *A. graveolens* (based upon plants cultivated in the Botanical Gardens of Kyoto University, Japan), it has been noted that metaphase plates show $2n=22$ (Figs. I-VI). Karyotype analysis of the two species showed that metaphase chromosomes comprise two sets of 11 chromosomes (Figs. II, IV-VI). Through observation of the karyotypes (Figs. II, IV) and idiograms (Figs. V-VI) indicates that there is a relationship of chromosome morphology between the two species.

From the fact that *A. Petroselinum* and *A. graveolens* have very similar morphological characters, but are different in the karyotypes, it may be assumed that they are closely related taxa. I wish, therefore, to express this relationship by placing them together in the same genus.

Involucel conspicuous; petals light green; node of radical leaf with 11 leaf gaps

.....*Apium Petroselinum*

Involucel wanting; petals white; node of radical leaf with 19 leaf gaps

..... *Apium graveolens*

Apium Petroselinum L. Sp. Pl. 264. 1753. Synonyms: *Apium crispum* Mill. Gard. Dict. ed. 8, Apium no. 2. 1768., *Petroselinum hortense* Hoffm. Gen. Umbell. 163.

* Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan. 京都大学理学部植物学教室

Table 1. Measurements of chromosomes in *Apium Petroselinum* and *A. graveolens*

Chromosome	A. Petroselinum (10=1.0μ)											A. graveolens (10=1.0μ)										
	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
Long arm	28	23	25	25	25	23	20	20	25			28	23	30	30	18	20					
Short arm	10+15	10+18	15	15	15	15	15	10	5	20	10	10+15	10+18	10	10	8	5	25	25	20	15	5
Total	53	51	40	40	40	38	35	30	30	20	10	53	51	40	40	26	25	25	25	20	15	5
Constriction	SM	SM	SM	St	SM	SM	SM	SM	SM			SM	SM	St	St	St	St					

1814, *Petroselinum sativum* Hoffm., op. cit., 177, *Petroselinum vulgare* Lag. Amen. Nat. 103. 1821, *Carum Petroselinum* Benth. & Hook. Gen. Pl. 1: 891. 1867, *Petroselinum crispum* (Mill.) Nyman ex Kew Hand-list Herbac. Pl. 3, 122. 1925.

Type locality: "In Sardinia juxta seaturigines." Distribution: Central and northern Europe; adventive in America and Japan. The plant is cultivated as a vegetable. The karyotype analysis of this species may be depicted as follows:

$$K(2n)=22=2^{CS}A^{SM}+2^{CS}B^{SM}+2C^{SM}+2D^{St}+10E^{SM}+4F$$

The lengths of the chromosomes of *A. graveolens* and *A. Petroselinum* are shown below:

Apium graveolens L. Sp. Pl. 264. 1753. Synonyms: *Apium integrilobum* Hayata, Mater. Fl. Formosa 126. 1911.

Type locality: Europe. This has a wide range in Europe, North Africa, America (adventive), and western Asia to northwestern India, also in Japan; the plant is cultivated for use as a vegetable.

B. Hayata recorded *Apium integrilobum* according to the specimen collected by U. Faurie 122, "in humidis Maruyama, Taipeh, Formosa". The writer, after careful examination of the isotype and many other specimens in the type locality of this plant, concludes that *A. integrilobum* is merely a specimen of *A. graveolens* grown in barren soil. The karyotype analysis of this species may be depicted as follows:

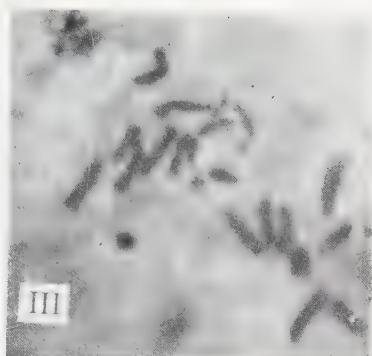
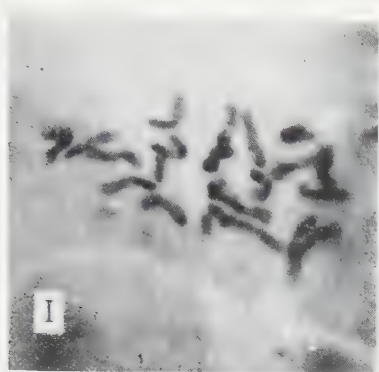
$$K(2n)=22=2^{CS}A^{SM}+2B^{SM}+8C^{St}+8D+2E$$

The writer expresses his cordial thanks to Prof. S. Kitamura and Prof. L. Constance (University of California, U.S.A.) for their guidance, and to Prof.

M. Shigenaga for his guidance in the cytological technique.

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II



IV



Figs. I-VI, Metaphase chromosomes in root-tip cells of *Apium Petroselinum* L. and *Apium graveolens* L. taken with a imm. obj. (n. A. 1.25) and a periplane oc. of C. Zeiss and magnified by $\times 2000$ (pretreated with 8-Oxyquinoline and stained with Orsein).

Figs. I-II, Somatic chromosomes ($2n=22$) of *Apium Petroselinum* L. Fig. I, Photomicrograph of metaphase plate. Fig. II, Schematic reproduction of Fig. I.

Figs. III-IV, Somatic chromosomes ($2n=22$) of *Apium graveolens* L. Fig. III, Photomicrograph of metaphase plate. Fig. IV, Schematic reproduction of Fig. III.

Fig. V, Chromosome idiograms of *A. Petroselinum* L.

Fig. VI, Chromosome idiograms of *A. graveolens* L.

Observational and Experimental Studies of Sensitive Plants V The Development of the Tannin Vacuole in the Motor Cell of the Pulvinus*

by Hideo TORIYAMA**

鳥山英雄: オジギソウの研究 V 葉枕の運動細胞に於けるタンニン液胞の発達

Received April 30, 1955

In the previous papers of the series of this investigation, the author has confirmed the presence of tannin vacuole in motor cells in *Mimosa* pulvinus, and described their morphological differences of the motor cells, before and after receiving stimuli (Toriyama 1953, 1954). In the present investigation, the development of tannin vacuole in the young plant was demonstrated in the fixed materials. The observation of the origin and development of tannin vacuole is of important significance to make clear the minute structure, together with the physiological function, of the vacuolar system of the motor cells. The writer wishes to thank Prof. Sirô Tarao who has given him much good advice and encouragement throughout this work. He also thanks Prof. Shun-ichirô Imamura of Kyoto University for his valuable suggestion.

Material and Method

The very young primary pulvinus of the seedlings of *Mimosa pudica* was mainly employed as the material. In comparison with this material, the pulvinus of *Robinia pseudo-Acacia* was also employed, thus the object of making sure of the development to tannin vacuole in the motor tissue has been attained. *Mimosa* was cultivated in the same manner which was described in detail in the previous paper (*l.c.*). For the observation of the motor cells before receiving a stimulus, the plants were exposed to ether vapour for 15 to 20 minutes. Thus, the pulvinus of these plants did not respond to any stimuli. To demonstrate the tannin vacuoles, the material was fixed with Kaiser's solution*** or Champy's fluid. The sections were cut 8 micra and were stained with Mallory's triple staining. The combination of Kaiser-Mallory's technic made it possible to demonstrate the origin of small vacuoles

* Contribution No. 17, from the Biological Section, Tokyo Woman's Christian College. This report was presented at the annual meeting of Kantô district of the Botanical Society of Japan, held in April 1955, in Tokyo. 科助成研. 課題番号 10769

** Biological Section, Tokyo Woman's Christian College

*** Kaiser's solution is composed of 10 gm. of sublimate, glacial acid 3 c. c. and distilled water 300 c. c.

by their brown staining, which is indicative of the first stages of the tannin vacuoles. This brown color by orange G is due to the appearance of the tannin substance which may be stained black by osmium salt.

Observations

Experiment 1: As stated in the foregoing paragraph, with a purpose to know the origin and the development of tannin vacuole in the motor cells, the author tried first to demonstrate the tannin vacuoles in the very young seedling by employing

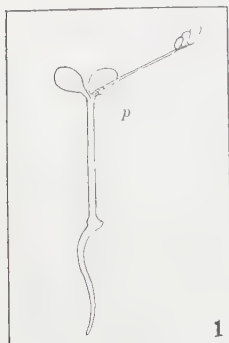
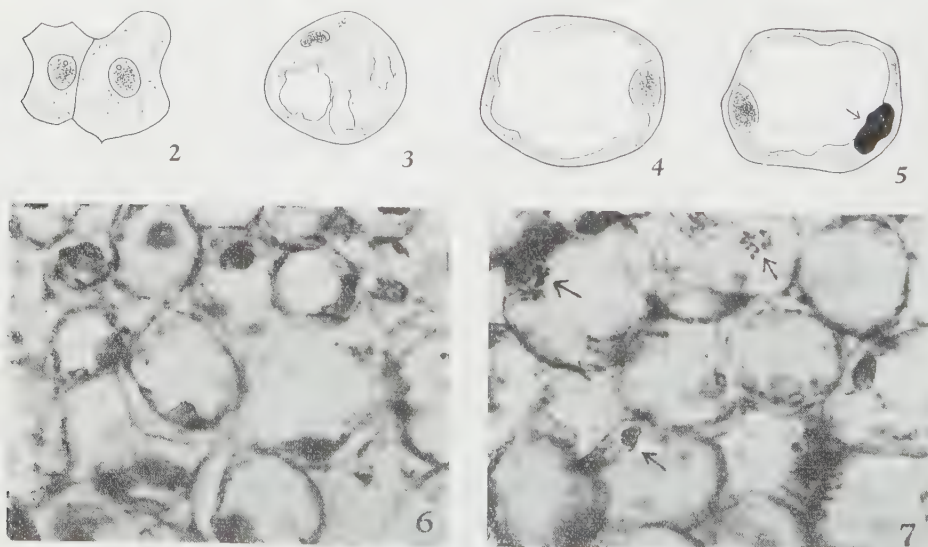


Fig. 1. Seedling of *Mimosa pudica*. p, young primary pulvinus.

Kaiser-Mallory's method. As shown in figure 1, very young seedlings which were cultivated in the laboratory room have leaves with petiole and primary pulvinus. Some of these plants, however, do not respond to the stimuli. In the motor cells of these plants the cytoplasm occupies, in most cases, the greater part of the protoplast, and there appear no vacuoles (fig. 2). In the plants which grow a little more than the foregoing material, the vacuoles with no tannin substance develop in the protoplast of each motor cell as shown in figure 3, while in some plants, very small tannin vacuoles appear in the protoplasm of the motor cells. Contrary to expectation, these plants with the tannin vacuoles did not respond to

the stimuli so well as the very young material without tannin vacuoles. From this fact it is extremely suggestive that the presence of the tannin vacuoles is by no

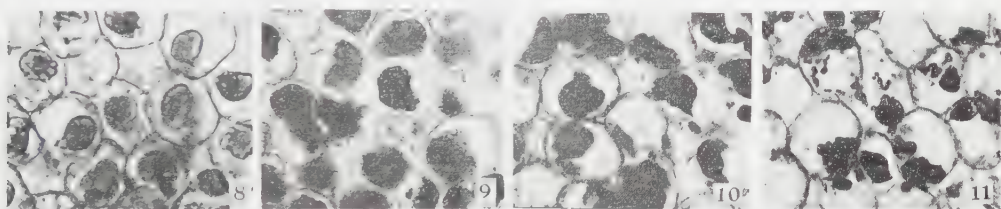


Figs. 2-7. Successive development of motor cell, $\times 800$. 2, very young motor cells 3, appearance of vacuole 4 and 6, protoplasm around large central vacuole 5 and 7, appearance tannin vacuoles. Arrows indicate the tiny tannin vacuole

means relevant to the seismonasty of the motor organ in the young plants.

Experiment 2: The plants were brought up in the pots exposed to the open air in the field. In the epidermal cells of the pulvinus and petiole in these plants, anthocyan is found to be formed. These plants respond to the stimuli. In some plants, as shown in figures 4 and 6, the cytoplasmic layer exists at the periphery of the cell surrounding the central vacuole, and does not contain the small tannin vacuole. In other plants, small tannin vacuoles, with almost the same size, appears in the protoplasm as shown in figures 5 and 7. There is no sign of change in the motor cell in both stages of plants before and after receiving stimuli. From these data it may be concluded that the tannin vacuole does not take part in the seismonasty of the motor cell of young material.

Experiment 3: Tannin vacuoles develop more and more as the plant grows. By Kaiser-Mallory's technic, the difference in motor cells before and after the bending movement in grown material can be observed as shown in figures 8 and 9. In

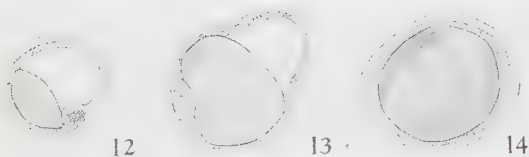


Figs. 8-11. Comparison before (8, 10) and after (9, 11) stimulus, $\times 480$. 8 and 9 are from the materials treated with Kaiser-Mallory technic; 10 and 11 are those fixed with Champy solution.

the motor tissue before the stimuli, the tannin vacuoles appear very cleary. The structural details in this phenomenon have been already brought up for discussion in the previous paper⁸⁾. By the fixation with Champy's fluid, more particular details are observable. In the anaesthetized plants the tannin vacuoles appear to be large and black with osmic acid as shown in figure 10. After the bending movement small globular vacuoles appear in the motor cell (fig. 11). These vacuoles and the small granules which are thought to be the result of disintegration of the former, are stained both black with osmic acid. This phenomenon is observable by other fixatives, *i. e.* by neutral formalin, or Buoin's solution.

Experiment 4: (Comparison between the development of tannin vacuoles in *Mimosa pudica* and that in *Robinia pseudo-Acacia*).

It is a well known fact that the leaflets of *Robinia pseudo-Acacia* display the "sleep- position" (nyctinastic movement) in the nocturnal condition. Tarao and Toriyama⁵⁾ and Toriyama⁶⁾ briefly reported the behavior of the tannin vacuoles in the motor cell of this plant in the day and the night time. The young pulvinus of *Robinia* was fixed and stained by means of the same technic as in the case of *Mimosa*. Figures 12, 13 and 14 show the successive stages of the development of tannin vacuole in the motor cells. In the large liquid vacuole appears the semicir-



Figs. 12-14. Successive developmental stages of motor cell of *Robinia pseudo-Acaica*. $\times 800$.

motor tissue of *Mimosa* in any of the plants. Thus, it may be regarded that the difference of the vacuolar system in the motor cell between *Mimosa* and *Robinia*.

Discussion

From the experience in the second work of the present series of investigations which deals with the modes of staining reaction of the tannin vacuole in the motor cells after Kaiser's fixative, it has been realized that the combination method of Kaiser-Mallory is the most suitable for the differentiation of the tannin vacuole and the cytoplasm.

It was observed that the protoplast of the young motor cell shows no tannin at a certain stage of the growth. The vacuoles are very well fixed in Kaiser's solution. These minute vacuoles develop more and more till they become a large liquid vacuole (figs. 3, 4). Through the same procedure, the origin of small tannin vacuoles appears clearly in young motor cells. Thus these two structures are distinguishable from each other by their staining properties and from their development. Went, Klercker and Lloyd* briefly reported the existence of two categories of vacuoles in the mature cells of numerous plants. Mangenot³⁾, also, could differentiate the tannin vacuole from the non-tannin vacuole of motor cell of *Berberis vulgaris* and *Mimosa pudica*. Therefore, it is unreasonable to regard the existence of two categories of vacuoles as an exceptional structure in the plant cells. Figures 15a and b show the structure of the typical motor cell before receiving a stimulus. This material was fixed with 10 per cent neutral formalin and stained with neutral red. This technic made it possible to demonstrate the tannin vacuole by its red staining, and the cytoplasm was observable clearly adjacent to the cell wall. As stated in the previous paper⁷⁾, it must be concluded that Aimi's work¹⁾ was insufficient from the cytological standpoint concerning the

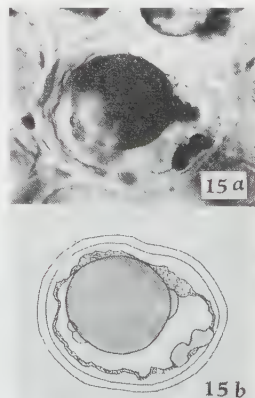


Fig. 15a, b. Typical motor cell of *Mimosa pudica* fixed with 10% neutral formalin and stained with neutral red, $\times 800$. b is the sketch from the photograph a.

* Cited from Guilliermond²⁾

motor cell of *Mimosa*. Indeed, in order to understand the cytophysiological mechanism of the bending movement, it is necessary to observe the minute structures precisely.

From the available data of experiments 1 and 2, it is concluded that the presence of tannin vacuole in motor cells is dispensable in the mechanism of the seismonasty. Concerning this, Molisch⁴⁾ stated briefly that the tannin vacuole has no direct connection with the sensitivity of the motor cells, but plays some rôle upon the regulation of turgor of the motor cells of *Mimosa*. The present author has the same opinion as that of Molisch. These problems should receive the earnest consideration of the physiologist. By Kaiser's fixative, a change in the tannin vacuole appears in the motor cell after receiving a stimulus, and by Champy's method numerous granules are observed inside the central vacuole. It seems that the tannin substance is mixed with the protoplasm or the some chemical substance in the large liquid vacuole. Perhaps such mixing is brought about by the change of the colloidal state of the protoplast of the motor cells. In any case this phenomenon seems to be the result of the bending movement. As the author⁹⁾ briefly stated, the potassium solution is issued from the motor cells into the intercellular spaces. These migrations of potassium may be, in the same way, caused by the mixing of tannin substances with the protoplasm in the motor cells.

It is a well known fact that the tannin substances are contained in the guard cells of the stomata, and in the motor cells of some plants*. The author's experiments in this work do not support the view that tannin vacuoles have a direct connection with the seismonasty of the motor cell of *Mimosa*. Nevertheless, as already stated, a large tannin vacuole always exists in the motor cell in the adult material. From this fact it can not be overlooked that the tannin vacuole might take a certain part in the seismonasty of the motor organ of these sensitive plants. The present author supposes that the tannin substance has, with all probability, a physiological significance for the regulation or maintenance of turgor of the motor cell. Further examination on the migration of substances, especially tannin substance and potassium in and out of the motor tissue may be critical studies to elucidate this problem from cytophysiology.

Summary

The available data from the present study concerning the development to the tannin vacuole in the pulvinus of *Mimosa pudica* may be summarized as follows.

1. By fixing the motor tissues with Kaiser's solution the tannin vacuole formation of all stages was demonstrated. The protoplast of young cell, which contains vacuole at first, begins to have a tiny original body of tannin nature at a certain stage.

* mentioned in Toriyama⁷⁾

2. The presence of the tannin vacuoles is by no means relevant to the sensitivity of the motor cell in young seedling.

3. As the result of the material confusion in motor cell, which may be caused by the bending movement, the morphological change in the tannin vacuole appears in the motor cell.

4. In the young pulvinus of *Robinia pseudo-Acacia*, tannin substance appears in the central vacuole of the parenchymatous motor cell, forming a vacuolar appearance. In the full grown pulvinus, the tannin vacuole finally fills up the whole central vacuole of the cell, leaving no trace of the latter.

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8. ———. Ibid. 19: 29-40 (1954a).
9. ———. Bot. Mag. Tokyo 67: 104 (1954b).

Cytological and Morphological Studies on the Gametophytes of Ferns IX The Polar Plasmolysis on Fern-prothallium (3)*

by Isami IGURA**

伊倉伊三美：羊齒類の配偶体に関する細胞学的並に形態学的研究 IX
羊齒類前葉体の有極性原形質分離 (3)

Received February 2, 1955

III The osmotic value, the isotonic and the permeability coefficient

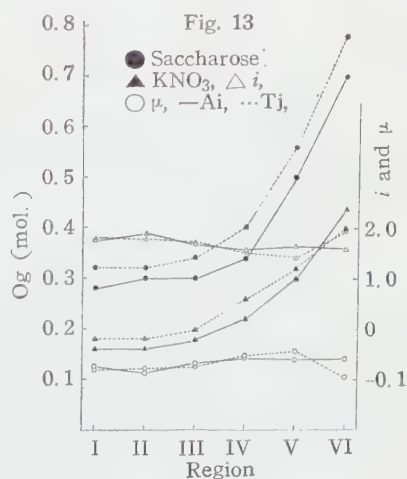
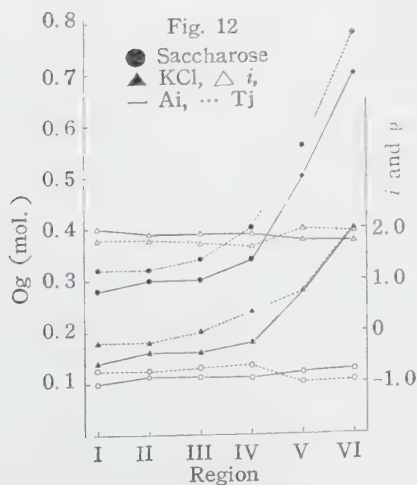
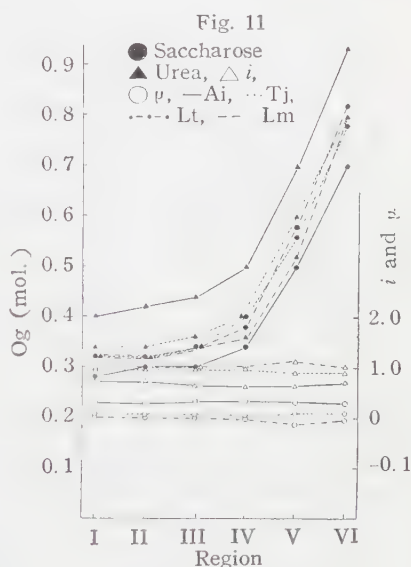
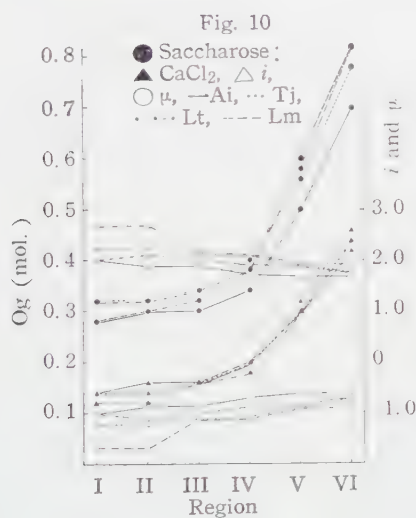
As for the osmotic gradients of saccharose in the cells of the prothallium which were in developing or in the adult stage, Gratzky-Wardengg⁸⁾ reported on *Nephrodium filix-mas* and *Struthiopteris germanica* and Reuter¹⁸⁾ studied also, by glucose, on *Dryopteris parasitica*. These authors, however, had not treated the isotonic and

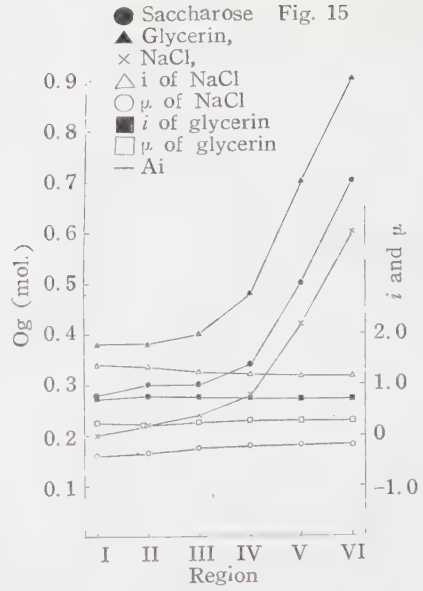
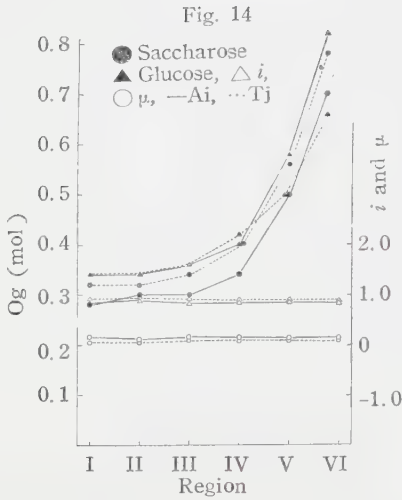
* The expences of this study were partly paid by the Grant in Aid for the Micellaneous Scientific Research from the Ministry of Education.

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the permeability coefficient. Using various reagents for four species of the fern-prothallia, the present writer decided the osmotic values by means of finding the incipient plasmolysis in graduated mol-solutions of the reagents, and then decided the isotonic and the permeability coefficient in detail at each region.

When the limit concentration (Og) of saccharose and the other plasmolyticum are C and C' respectively, the isotonic coefficient (i) is calculated from the ratio of C to C', viz. i is led from the formula of De Vries, $i = \frac{C}{C'}$ and the permeability coefficient (μ) is acquired from the formula, $\mu = 1 - \frac{C}{C'}$ which was given by Tröndle^{20,21}. In addition to these data, the atmospheres were calculated from the limit concentration of saccharose by the formula of van't Hoff, $P = \frac{n}{v} RT$. The increasing gra-





Figs. 10-15. The osmotic value (O_g), i and μ of the various reagents and the correlations among them in the fern-prothallium. This experiment was made from January to May, 1954. Ai *Asplenium incisum* Thunberg, Tj *Thelypteris japonica* Ching, Lt *Leptogramma totta* J. Smith, Lm *Leptorumohra Miqueliana* H. Ito.

dients of the osmotic values were also shown in figures which were the percentages of the increased value at each region to the value at Region I. Of these results, O_g , i and μ will be described and the correlations among these results will be given by graphs in Figs. 10-15.

The cells of the basal protonema possess the low limit concentrations, this is, the low osmotic values; on the other hand, those of the apical meristem show the high. Namely, the osmotic value is lowest at Region I, or I and II, and is highest at VI. In other words, the osmotic value is low in the cells of basal pole formed earlier than those of apical pole, and the latter have the high osmotic values. It is interesting that the gradient of the osmotic value is found between the basis and the apex of the prothallium, and in all cases as the region approaches the meristem region from the protonema one, the osmotic value increases by degrees. As to the ratio (gradient) of increase, the difference between each region and Region I or between the neighbouring region each other is not equal and the nearer the region approaches the meristem, the greater the gradient becomes.

The osmotic values of the electrolytes were lower than those of the nonelectrolytes in fern-prothallium also, for instance, while the osmotic value of KCl was 0.28 mol., that of urea was 0.7 mol. at Region V in the prothallium of *Asplenium incisum* Thunberg, though some electrolytes such as $AlCl_3$, $MgCl_2$, and Na_2SO_4 presented more or less high osmotic values.

The isotonic coefficient is apt to decrease and the permeability coefficient gene-

rally shows the tendency to increase as the region comes near towards the apical pole, although sometimes this phenomenon is disturbed at some regions. The fact that the permeability coefficient of saccharose to CaCl_2 is negative (viz. $\mu < 0$) seems to be explained that the substance of the latter penetrates into the cytoplasm less than that of the former. The examples like this are found also in KCl , KNO_3 , and NaCl . The atmosphere of the prothallial cell at each region calculated from the osmotic value of saccharose by the formula of van't Hoff is naturally lowest at Region I, or I and II, and highest at VI. The extracts of these results were drawn in graphs (Figs. 10–15) as above-mentioned, and though there were some differences, the osmotic values (O_g), i and μ of all species were almost in parallel in their increase or decrease.

IV The permeability of reagent (Deplasmolysis)

The purpose of this investigation was to find out the characterized permeability in the young and the old cell of the prothallium. The plasmolytica to which the prothallial cytoplasm showed the high permeabilities and in consequence the marked deplasmolyses, are urea and secondly glycerin, while with respect to glucose and KNO_3 , especially the other reagents the good results of deplasmolysis could not be

obtained. Concerning the results of the present experiment, the writer treated chiefly urea and glycerin as Reuter¹⁸⁾ pointed out. The durations in which the deplasmolysis finished in urea- and glycerin-solutions were estimated, and their extracts will be reported in Fig. 16.

Judging from the data in Figs. 10–15, it is found that Region I has the lowest permeability and V the highest, because the duration of deplasmolysis is longest at Region I and the progressive decreases is recognized according as the region comes near the meristem. This fact is contrary to the result on the

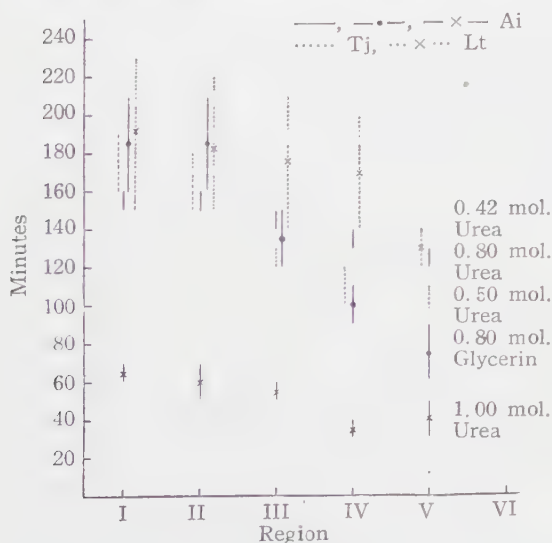


Fig. 16. The durations of deplasmolysis in urea- and glycerin-solutions in the fern-prothallium

duration of plasmolysis. In reference to the correlation between urea and glycerin, the following fact was found in 0.6 mol. urea- and glycerin-solutions in *Asplenium incisum* Thunberg (Fig. 16), that is, urea is more permeable than glycerin to the cells of the basal pole (neighbourhood of Region I) and on the contrary glycerin is more permeable than urea to the cells of the apical pole (neighbourhood of Region

V). Therefore, it is presumed that the cell in the neighbourhood of the protonema (older than the meristem portion) is the urea-type and the cell in neighbourhood of the meristem (younger than the protonema portion) is glycerin-type.

Table II. The deplasmolys's in KNO₃-solution after 24 hours in the prothallium of *Thelypteris japonica* Ching

Mol.	Regions						Remark
	I	II	III	IV	V	VI	
0.22	±	+	+				III-17; 17°.5, II°C
0.28	±	±	+	+			
0.32	±±	±±	±	+	+		
0.24	±±	±±	±	+	±		

The deplasmolysis of the prothallial cell in KNO₃-solution in a certain duration was that at the region near the meristem the deplasmolysis occurred, but in the one far from it the slight deplasmolysis arose or not (Table V). In short, at the neighbourhood of the apical pole the deplasmolysis occurs earlier than at that of the basal pole in the longitudinal polarity and though no correct observation was got in some cases, the same results seemed to be given also in the tangential or the radial polarity.

(to be continued)

受光量と土壤水分の量とがシラカシ苗の 耐陰性におよぼす影響

高原末基*・川名 明**・丹下 勲**

Suemoto TAKAHARA, Akira KAWANA and Isao TANGE:
Influence of Light Intensity and Soil Moisture on the Shade-Endurance
in the Leaves of Shirakashi-Seedlings
(*Cyclobalanopsis myrsinaefolia* Oerst).

1955 年 4 月 5 日受付

I 緒 言

さきに著者の一人¹⁾はスギ、ヒノキをもちいて生育光度、土壤水分などの生活環境がそれらの補整点にどのような影響をあたえるかをしらべた。

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本実験ではそれと同様な方法でシラカシの苗をもちいて実験をおこない、その補整点の変化をしらべ、さらにクロロフィル含有量、全窒素量および無機要素量などを調査した。
実験に当り御便宜を与えられた東大千葉県演習林職員各位に心から感謝する。

II 実験方法

a) 供試苗の処理： 実験にもちいたシラカシ苗は3年生で、東京大学千葉県演習林武者土苗畑で育成したものである。試験区は全陽光乾燥、同湿潤、庇陰乾燥および同湿潤の4区とし、演習林天津溝内のガラス室内に設け、各区ともそれぞれ1鉢1本宛植栽し、1区6鉢について実験をおこなった。

土壌は天津土壌（密状態における含水量 36.6 %）をもちいた。庇陰区は関係光度が 13% になるようにヨシノで遮光を施し、土壌水分は乾燥区が含水量の 25%, 湿潤区は 85% に保つように灌水をおこなった。

供試苗は 1952 年 4 月 19 日に採取、4 月 22 日に鉢植えとし、6 月 18 日に各区別に光度および土壌水分の処理をおこなった。このような処理の下で約 14 ケ月間生育させたのち、1953 年 8 月 13 日にそれぞれ当年生葉について、補整点、クロロフィル含量を測定し続いて全窒素および灰分の分析をおこない、それぞれ 5% 以下の危険率で差の検定をおこなった。

b) 補整点の測定： 補整点の測定は電気式微量天びかゝ自動測定装置^{11) 12)} をもちいた。光源は 1 KW の投光器をもちい、同化箱中の温度は 25°C に保持した。

c) クロロフィルの定量： メタノール・ベンゼン法で定量した^{11) 13)}。

d) 全窒素および灰分分析： 全窒素は KJEL-

て、P₂O₅ より間接的に定量した。K₂O はヘキシル・ノトリウム重量法によつた。

III 実験結果

実験結果を第 1 表および第 2 表に示す。

a) 補整点、全陽光、庇陰区とも乾燥状態のものが湿潤のものより高く、また乾燥区では庇陰されたものが低く、全陽光湿潤区と庇陰湿潤区との間には差があるとはいひきれない。

b) クロロフィル含有量、それぞれ同じ光度のもとでは乾燥区と湿潤区との間に差がないが、それぞれ乾燥および湿潤の両区では全陽光下におけるものが、庇陰下におけるものよりクロロフィル含有量が低い。

c) 全窒素および無機要素含量、全窒素は庇陰下に多く乾燥および湿潤区では、乾燥区よりも湿潤区に多い傾向がある。灰分は全陽光下の葉が庇陰下のそれより多く、乾燥区と湿潤区には差がない。SiO₂ もがいして灰分と同様の傾向を示すが、ただ乾燥区より湿潤区に幾分多い傾向がみられる。P₂O₅ も全陽光下に多く、全陽光下では湿潤

第 1 表

試 験 区	補 整 点 Lux	葉 緑 素 生重当り%
全 陽 光 乾 燥	527	0.248
全 陽 光 湿 潤	177	0.252
庇 陰 乾 燥	135	0.683
庇 陰 湿 潤	100	0.615

試 験 区	Total-N %	Ash %	SiO ₂ %	P ₂ O ₅ %	CaO %	MgO %	K ₂ O %	CaO/MgO	CaO/K ₂ O
全 陽 光 乾 燥	1.46	9.42	3.06	0.46	2.67	0.88	1.09	3.03	2.45
全 陽 光 湿 潤	1.66	9.84	3.14	0.58	2.58	0.81	1.34	3.19	1.93
庇 陰 乾 燥	2.68	8.66	2.16	0.36	2.43	0.94	1.29	2.59	1.90
庇 陰 湿 潤	2.90	8.62	2.25	0.34	2.10	0.67	1.42	3.13	1.48

第 2 表 Total-N 以下は絶乾重当り

DAHL 法で定量した。灰分は王水で分解して残渣を SiO₂ として重量法で定量し P₂O₅ は PENBERTON-石橋法、CaO、MgO は pH 4.0 の醋酸一醋酸アンモン Buffer で二、三酸化物を磷酸塩として除去したのち、CaO は過マンガン酸カリ容量法、MgO は磷酸マグネシウム・アンモニウムとして分離し、これを P₂O₅ の定量法に転換し

区に多く、庇陰下では乾湿による差がないようである。CaO は全陽光下に多いが、乾燥区と湿潤区では乾燥区に多く含まれる傾向があり、とくに庇陰下ではその差が大きい。MgO は光度による差が明らかでなく、乾燥区は湿潤区より大で、とくに庇陰湿潤が小さい。K₂O は庇陰下に多く、湿潤区におけるものが乾燥区より多く含まれてい

る。 $\text{CaO}/\text{K}_2\text{O}$ は湿潤区が乾燥区よりも低く、陽光下では庇陰下よりも高いが CaO/MgO は湿潤や受光量の増加で高くなっている。

IV 考 察

著者の一人¹⁾はさきに補整点は生育光度と土壤水分とに密接な関係があることを認めた。本実験ではさきにおこなったスギおよびヒノキの場合と光度および土壤水分処理条件は同一である。しかし処理期間が前者に比較して長く、かつ供試葉はスギおよびヒノキの庇陰された葉そのものであるのに対し、シラカシでは庇陰下に発生した葉である。シラカシの補整点はがいしてスギおよびヒノキよりかなり小さいが、この点に関しては庇陰処理期間の長短ならびに供試葉の年令の差^{5) 9)}などを考慮に入れる必要があろう。またシラカシの補整点は光だけでなく土壤水分の影響も大きい。スギおよびヒノキの場合と異なり庇陰下の湿潤区のものが乾燥区より補整点が低いことは、さきの全般に補整点が低いことと相俟つてシラカシの葉が低光度に適応して光合成能力を高め得る性質が強いことを示している。

クロロフィル量もまた庇陰下でおおくなることはさきに認めたが¹⁾、シラカシも庇陰下の葉に多く補整点の低下とともに生理的陰葉に転移したことを示している。クロロフィル量は受光量の多少に関係するほか、その他の環境条件にも支配されると思われるが、本実験の範囲内では土壤水分の変化によるクロロフィル含量に差を認めることはできなかった。

陽葉または陰葉あるいは樹木の葉の年令の相違によつて葉の灰分含量^{2) 5) 9) 11)}が異なり、また落葉前の葉の塩類含量の変化が知られ^{4) 10)}、葉の無機要素含量はその生理的状态を示すものと考えられる³⁾。本実験では灰分、 SiO_2 は生育光度が高いものに多く土壤水分の影響はがいして少い。

全窒素は光が少いものに多く、湿潤区に多いが P_2O_5 は光が多いものに多く、全陽光下では湿潤のものにおおい。また MgO は乾燥区に多く、湿潤下では陽光区に、乾燥下では庇陰区におおく、蒸散の困難な区に小さい傾向を示している。窒素は蛋白、酵素として、 P_2O_5 は蛋白ばかりでなく高エネルギー磷酸として生命活動に大きな働きをなし、 MgO はクロロフィル、あるいは酵素結合と

して働き、 P_2O_5 の転移にも大きな役割をはたしている¹⁾。

CaO 、 K_2O 、 MgO などを $\text{CaO}/\text{K}_2\text{O}$ 、 CaO/MgO の比率からみるとともに、光に対して陽光区に大であるが、水分に対しては前者は湿潤で小になり後者は大になる。K は細胞膠質の解膠に働き水分の動きをおそくし、Ca はこれと逆の動きを有すると考えられるので $\text{CaO}/\text{K}_2\text{O}$ は水分代謝のために適応していると考えられる⁷⁾、これに対して CaO/MgO が水分の動きのおそいものに小さいことを示している。

これらの関係からみると庇陰湿潤は P_2O_5 、 MgO が少く生命活動が小さく^{1) 3)}、N が多く P_2O_5 が少く耐病性が弱くなっている^{3) 14)} ことが想像される。

これは要するに生育光度の高いもの、土壤水分の低いものほど補整点が高く、庇陰湿潤区は補整点、クロロフィル、全窒素、 K_2O などから考えるとよく環境に適応してその低光度における光合成能力を高めていることを示すが^{4) 6) 11)}、庇陰下のは耐病性が弱く、ホルドー液による消毒をおこなつたが庇陰湿潤区では、処理中一部(2本)枯死したものがみられ、個体の適応能力の差もあると思われ、 P_2O_5 の減少、 MgO の減少などが示すように生命活動の衰えを示し、N の増加、 P_2O_5 の減少から耐病性の低下が考えられる。

季節によつてその影響の程度に差のあることも考慮する必要があるが、本実験ではシラカシ苗について生育光度および土壤水分の変化が耐陰性に対し、どのような生理的变化に影響をおよぼすかを推察することができる。また植物の耐陰性を論ずる場合に補整点が生育環境で大きく動くこと、光合成能力は低光度によく適応してもその他の生命活動、耐病性などの見地から検討を要するものと考えられる。

V 摘 要

全陽光乾燥、同湿潤、庇陰乾燥ならびに同湿潤の4区をガラス室内に設置し(庇陰区は関係光度13%、土壤水分は湿潤区が容水量の85%、乾燥区が25%とした)、シラカシの3年生苗をこれらの試験区で栽培した。これらの処理をおこなつて約14ヶ月後に当年生葉の補整点、クロロフィル含量、全窒素含量、無機要素含量などをしらべてつぎの

結果を得た。

1) シラカシの補整点は光強度だけでなく、土壌水分の影響を受けて庇陰下で低くなる。光度の影響は乾燥区にとくに著しい。同じ光度では湿潤区の補整点が低い。

2) 葉のクロロフィル含量は庇陰下に高く、土壌水分の影響は本実験の範囲内では認められない。

3) 葉の全窒素や K_2O の含量は庇陰下に高く、一方、 SiO_2 , P_2O_5 は日光下の草に多く含まれている。湿潤区の葉には全窒素、 SiO_2 , K_2O が多いが、乾燥区では CaO , MgO が多い。

低光度での光合成能力は庇陰によつて高まり、水分によつて塩類のバランスが保たれるが、庇陰下では生命活動が衰えているようである。

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Résumé

Compensation point, content of chlorophyll, total nitrogen and mineral elements in the leaves of Shirakashi-seedlings (3 years old) cultured under various conditions of light intensity and soil moisture were surveyed. Four experimental plots were established in a green house; viz, a dry and a wet plot in the light and in the shade respectively. The seedlings were cultured in those plots, for about 14 months (relative light intensity in the shade plots being about 13%; soil water in the wet plots being 85% of the capacity, soil water in dry plots being 25%).

The results obtained are summarized as follows.

1) The compensation point of the leaves of Shirakashi was not only affected by the light intensity, but also by the soil moisture. By the former it was remarkably influenced in the dry plot, while scarcely in the wet.

Under the same condition of light intensity, the compensation point was strikingly influenced by the soil moisture, namely, it was lower in the wet than in the dry.

2) Chlorophyll content of the leaves was higher in the shade plot than in the light, and it was not affected by the soil moisture as far as this experiment was concerned.

3) The contents of total nitrogen and K_2O were larger in the shade plot, on the other hand, ash, SiO_2 and P_2O_5 were contained more in the leaves of the sun plot.

Relatively high contents of total nitrogen, SiO_2 and K_2O were observed in the leaves of the wet, while the contents of CaO and MgO were smaller in the wet than in the dry.

蘚類数種の染色体X

タマゴケ科の核型と性染色体*

矢野孝二**

Koji YANO: On the Chromosomes in Some Mosses. X.
Karyotype and Sex Chromosome of *Bartramiaceae*

1955 年 5 月 18 日受付

タマゴケ科 (*Bartramiaceae*) のうちサワゴケ属 (*Philonotis*) の染色体に関しては僅かに 1 種 *P. fontata* で Heitz¹⁾ が $n=7-8$, Vaarama⁵⁾ が 6 II を報告したに過ぎない。筆者は今回本

属の 7 種及び同じくタマゴケ科のタマゴケ属 (*Bartramia*) の 2 種の染色体を観察し、主としてそれ等の核型を定めた。その結果サワゴケ属の 4 種では性染色体を明らかにし、更にそのうちの

Table I

植 物 名	性別	染色体数 (n)	核 型	採 集 地 ²⁾
<i>Philonotis falcata</i> (Hook.) Mitt.	♀ ♂	6 6	V(X)+3V+J+m(h) V(Y)+3V+J+m(h)	金 谷 村
<i>P. japonica</i> (Schimp.) Par.	♀ ♂	6 6	V(X)+3V+J+m(h) V(Y)+3V+J+m(h)	青海黒姫山
<i>P. carinata</i> Mitt.	♀ ♂	6 6	V(X)+3V+J+m(h) V(Y)+3V+J+m(h)	青海黒姫山
<i>P. lancifolia</i> Mitt.	♀	6	V(H)+3V+J+m(h)	雨 飾 山
<i>P. seriata</i> Mitt. ¹⁾		6	V(H)+3V+J+m(h)	池 之 平
<i>P. Turneriana</i> (Schwag.) Mitt.	♂	6	V(H)+3V+J+m(h)	蓮 華 温 泉
<i>P. socia</i> Mitt. (一倍体)	♂	6	V(X)+3V+J+m(h)	市 振 青海黒姫山
同 上 (二倍体)	♂ ♀	6 12	V(X)+3V+J+m(h) 2V(X,Y)+6V+2J+2m(h)	
<i>Bartramia pomiformis</i> (L.) Hedw.	♀	8	V(H)+3V+2J+m+m(h)	妙 高 山
<i>B. crispata</i> Schimp.	♀	8	V(H)+3V+2J+m+m(h)	春 日 山

註 1) 性別不詳 2) すべて越後

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1 種では種内倍数体をも見出したので、以下これ等の結果について報告する。

研究に用いた蘚の種名、性別及び採集地は Table I の如くであり、固定並びに染色法は前報告の場合と同様である。

観 察 結 果

1) *Philonotis* 属の核型及び性染色体

Table I に示す如く、観察された *Philonotis* 属の 7 種 (*P. falcata*, *P. japonica*, *P. carinata*, *P. lancifolia*, *P. seriata*, *P. Turneriana*, 及び *P. socia* の一倍体) の染色体数は何れも $n=6$ で、その核型はすべて $4V+J+m$ である。そしてそれ等の染色体のうち最大の V と最小の m とは他の多くの植物の場合と同様に異質染色体で、それぞれ H 及び h で示される。更にこれ等 7 種のうち *falcata*, *japonica*, *carinata* 及び *socia* の一倍体の 4 種では雌雄の染色体を比較したが、その H は雌雄の間で異常凝縮其他に相違があつて、これは性染色体であることがわかつた。そしてこれ等 4 種の性染色体の凝縮は互によく似てい

る。それで以下主として *P. falcata* について、各染色体の形態並びに性染色体を報告する。

P. falcata (Figs. 1-12) の雌性の性染色体 X は前述の如く核板中最大の V 形染色体で染色体の中央部に一次狭窄が認められる。更に一腕 (左腕)* の尾部及び他腕 (右腕)* の中央部にそれぞれ 1 個づつ計 2 個の二次狭窄が認められる。後者即ち右腕の二次狭窄は微弱であつて時には認め難い場合もあるが、その場合でも右腕はこの二次狭窄の部分で折れ曲り、その為に X の全形は S 又は W 字形をなして現われることが多い。 X の異常凝縮は一部分にみられ、それは右腕の二次狭窄より以下の尾部約半分と、左腕の尾端の小部分である (Figs. 3, 4)。

Y も亦雌性核板中最大の V 形染色体で、その大きさは X とほぼ同大である。然し二次狭窄は X とは異り、その数は 1 個で、それは左腕の尾部に認められ、右腕には X に見られた様な狭窄が認められない。更に Y は異常凝縮においても X と異なる。即ち Y の右腕はその全部が異常凝縮を示し、 X の右腕の半分が異常凝縮を示すのとは大に異っている。但し Y の左腕端の異常凝縮は X と同様である (Figs. 7, 8)。従つて Y の方が異常凝縮部が多く、その為前期ないし休止期において雄性核の染色質塊は雌性核のそれよりも常に大きい。

其他の 5 個の染色体は雌雄全く共通である。それは 3 個の V , 1 個の J , 1 個の m より成る。この m は既報の酢の場合と同様異質染色体 h である。そして、その異常凝縮塊は休止期の核中でしばしば仁の中心部に認められる。

以上の結果から本種の核型は次の如く示される。

$$\text{♀ } K(n)=6=V(X)+3V+J+m(h)$$

$$\text{♂ } K(n)=6=V(Y)+3V+J+m(h)$$

本種の子囊体の核板には $2n=12$ の染色体が認められる。即ちこの核板では期待通り、2 個宛が全く同形同大の 5 対の常染色体と、 X と Y とが認められた (Fig. 10)。この場合は同一核板中に X と Y とが見られるので両者の形態の比較が容易であるが、これ等の形態は上記の配偶体で観察された XY の形態と全く一致した。また子

* 以下 H では、尾部に二次狭窄をもつ腕を左腕、他方を右腕とする。



Figs. 1-12. *Philonotis falcata* の染色体と異常凝縮

Chromosomes and heteropycnosis of *Philonotis falcata*.

1-4. ♀ gametophyte 5-8. ♂ gametophyte 9. Serial alignment of the chromosomes shown in Figs. 1, 5 10. Sporophyte 11, 12. Meiotic chromosomes at the 1st metaphase and anaphase of SMC's $\times 1330$

囊体の核には XY 及び 2 個の h に由来する計 4 個の異常凝縮塊が見られる。このうち 2 個の h の異常凝縮塊には何等の差が認められないが、X と Y とのそれには配偶体で認められたと同様な明瞭な差異が見られた。

胞子母細胞の減数第一分裂中期では 6 II が見られる (Figs. 11, 12) これ等のうちの 1 個は他の 5 個と接合状態が異りしばしば特異な形態を示す。即ちこの二価染色体は 2 染色体が一部分でのみ接合している。尚これは後期で他の 5 個に先ん



Figs. 13-26. *Philonotis japonica* 外 6 種の染色体と異常凝縮

Chromosomes and heteropycnosis of *Philonotis japonica* and other six species.

13-18. ♀ (13, 14) and ♂ (16, 17) gametophytes and sporophytes (15, 18) of *P. japonica*

19, 20. ♀ (19) and ♂ (20) of *P. carinata*
21. *P. lancifolia* (♀). 22 *P. seriata*. 23. *P. turneriana* (♂)

24, 25. *Bartamiamia pomiformis* (♀) 26. *B. crispata* (♀) ×1330

じて分離し始めるが、両極に達するのは他よりも多くの場合遅れる。この二価染色体は X-Y の対合と推定される。然し両極に向う X 及び Y の形態及び大きさの間には可視的な差異は認められなかった。他の 5 個の二価染色体は何れも接合が完全であつて、後期では何れも同形同大の染色体に分離しほぼ同時に両極に達する (Fig. 12)。

P. japonica, *P. carinata* 及び *P. socia* の一倍体でも雌雄の配偶体の染色体の比較を行い、特に *japonica* では子囊体の染色体をも観察した (Figs. 13-20, 27-34)。その結果これ等 3 種でも性染色

体を発見した。而してその性染色体の形態、異常凝縮の状態、及び他の普通染色体の形態は *falcata* のそれ等と一致した。従つてここにはこれ等の記述を省略する。

P. lancifolia では雌株、*P. Turneriana* では雄株、*P. seriata* では性別不詳の配偶体について観察を行つた。これ等の核型も *falcata* 等のそれと同様であつた (Figs. 21-23)。

2) *Philonotis socia* Mitt. の種内倍數体

本種には前記 $n=6$ を示すものの他に $n=12$ の種内二倍体が見出された (Figs. 27-38)。

上記の如く一倍体は雌雄異株であつたが、二倍体は雌雄同株である。この二倍体の最大の 2 染色体は何れも V 形の異質染色体 H である。此の両 H は形態及び異常凝縮性に於て互にやや異り、両者の間には上記一倍体の性染色体 X 及 Y 間に認められたと全く同様な差が見られる。従つてこれ等 2 個の H は、その形態的特徴及び性分化に於て一倍体が雌雄異株、二倍体が雌雄同株である事実から推察して、それぞれ性染色体 X 及び Y であろう。



Figs. 27-38. *Philonotis socia* の一倍体と二

倍体の染色体と異常凝縮
Chromosomes and heteropycnosis in mono-
ploid and diploid plants of *Philonotis socia*

27 30. ♀ gametophyte of monoploid

31-34. ♂ gametophyte of ditto

35-38. Gametophyte of diploid (♀).
×1330

その他の 10 個の染色体のうち最小の 2 個は異質染色体 h であるが、此の両者の形態及び異常凝縮性は一倍体の h と全く同様であり、亦此の両者間には何等の差が認められない。他の 8 個の常染色体は 6 V, 2 J で、これ等は一倍体の 4 個の常

染色体と同形の 2 組の染色体に分けられる。即ち二倍体の染色体は殆んど全く一倍体の雌雄の染色体組を合せたものに一致する。従つてその核型は次の如く示されるべきものであろう。

chum)¹⁰⁾, ハイゴケ科 (*Hypnaceae*) のハイゴケ属 (*Hypnum*)¹⁴⁾, 更に本報告のタマゴケ科 (*Bartramiaceae*) のサワゴケ属 (*Philonotis*) で性染色体を発見した。これら諸属の性染色体を比較す

Table II. *Philonotis socia* の一倍体と二倍体の細胞表面積の比較
(Comparison of the surface area of cells between the monoploid and the diploid plants in *Philonotis socia*)

	Monoploid		Diploid		M ₂ /M ₁
	n	M ₁ ± m	n	M ₂ ± m	
Leaf cells (μ ²)	225	233.4 ± 5.26	225	362.3 ± 8.33	1.55
Capsule wall cells (μ ²)	225	486.4 ± 11.20	225	689.5 ± 18.30	1.42

$$\phi \quad K(n)=12=2V(X, Y)+6V+2J+2m(h)$$

Table II は本種の一倍体と二倍体の細胞の表面積の比較である。この表から同属である様に二倍体は一倍体に比し著しい巨大化を示している。然し両者の間には性分化の差以外は質的な差は見られない。

3) *Bartramia* 属 2 種の核型

研究された本属の 2 種 (*B. pomiformis*, *B. crispata*) は何れも雌雄同株である。このうち *B. pomiformis* の染色体数に関しては Heitz¹⁾ が $n=7-8$, 栗田²⁾ が $n=8$ と報告した。筆者は本属の核型を定むる目的を以て上記 2 種の配偶体の染色体を観察した。その結果両種の染色体数、核型は全く同様で次の如く示される。

$$\phi \quad K(n)=8=V(H)+3V+2J+m+m(h)$$

即ち染色体数は栗田の報告と一致した。両種の染色体で特に他と異つてゐるのは次の二種である。即ち、(1) 最大の染色体 (V) は異質染色体 H であるが、これの異常凝縮を示す部分は右腕の端のみであつて他属に比して著しく異常凝縮部が少い。また、(2) 微小な m が 2 個見られるが、h はそのうちの 1 個のみであつて他の m は異常凝縮を示さない。尚後者は前者 (h) に比して幾分大きい。

考 察

筆者はスギゴケ科 (*Polytrichaceae*) の 3 属、即ちスギゴケ属 (*Polytrichum*)⁹⁾, ニラスギゴケ属 (*Pogonatum*)¹⁰⁾, タチコケモドキ属 (*Oligotri-*

ると (1) スギゴケ科 3 属では XY 間に大きさ及び形態の相違があり、X は Y よりやや大きい。それは X の右腕は Y のそれよりやや長い為である。更に X の右腕には中央部に二次狭窄が見られるが Y には認められない。(2) ハイゴケ属では XY 間に大きさ及び形態の相違は認められない。ただ異常凝縮性に相違が認められる。(3) サワゴケ属では XY 間に大きさの差はないが、形態に於て前記スギゴケ科 3 属の場合の如き相違があり、X の右腕には二次狭窄があるが Y にはこれが認められない。異常凝縮は前記ハイゴケ属と同様な差異が認められる。

以上の如く各属の性染色体には次の如き共通性及び特性が見られる。(1) XY は常に異質染色体 H であり、それは核板中最大か又は著しく大きな染色体である。(2) XY 間に大きさの差がある場合 (スギゴケ科 3 属) でもその差は小さい。(3) XY の左腕には常に尾部に二次狭窄がある。(4) XY 間に形態的相違がある場合はそれは右腕であつて、X にはこれに二次狭窄があり Y にはない。(5) XY 共に異常凝縮を示す部分は主として右腕であり、属によつては (スギゴケ科 3 属, サワゴケ属) X に比して Y の方がこれを示す部分が大きい。この様に既知の蘚類の性染色体の分化には種々なる程度があることがわかる。

筆者は既に他の多くの蘚類で核板中最大の染色体が異質染色体 H であることを報告しているが、これ等の多くでは未だ雌雄の II の間に上記の如き形態的な分化が見出されなかつた^{6, 7, 8, 11, 12, 13)}。然しこれ等の H も形態及び異常凝縮性に於て上

記各属の性染色体と多くの類似性をもっている。

従つておそらくこれ等の H は性染色体と相同なもので、性染色体は特に H の形態的分化が進み、雌雄の間で差異が生じたものと考えられる。既に研究された蘚類では何れも核板中最小の染色体は異質染色体 h であるが、この染色体には未だ雌雄の間で差が認められない。長野 (1954) は苔類のケゾニゴケの休止核の仁内に見られる染色質塊 (Nukleolus) は h に由来すると報告した。筆者も多くの蘚類で h が Nukleolus となることを見ており、今回の材料でもこのことが認められたから、蘚類に於ても h はいわゆる Nukleo-

linus-Chromosomen であろう。

Philonotis socia では種内倍数体が見出された ($n=6, 12$)。而して一倍体は雌雄異株、二倍体は雌雄同株である。又二倍体の染色体は一倍体の雌株と雄株の染色体を併せたものに相当する。これと全く同様な関係は既に *Thamnium Sande*⁸⁾ *Brachythecium Brotherii*¹¹⁾ の種内倍数体でも報告され、おそらく二倍体は一倍体の子嚢体から Apospory によつて導かれたものであろうと推定されている。今回の *Philonotis socia* の二倍体の成立についても同様に考えられる。

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Résumé

1) The chromosome numbers and the karyotypes of genus *Philonotis* and genus *Bartramia* studied are as follows;

<i>Philonotis falcata</i> (Hook.) Mitt	♀ $K(n)=6=V(X)+3V+J+m(h)$
	♂ $K(n)=6=V(Y)+3V+J+m(h)$
<i>P. japonica</i> (Schimp.) Par.	♀ $K(n)=6=V(X)+3V+J+m(h)$
	♂ $K(n)=6=V(Y)+3V+J+m(h)$
<i>P. carinata</i> Mitt.	♀ $K(n)=6=V(X)+3V+J+m(h)$
	♂ $K(n)=6=V(Y)+3V+J+m(h)$
<i>P. lancifolia</i> Mitt.	♀ $K(n)=6=V(H)+3V+J+m(h)$
<i>P. seriata</i> Mitt.	$K(n)=6=V(H)+3V+J+m(h)$
<i>P. Turneriana</i> (Schwag.) Mitt.	♂ $K(n)=6=V(H)+3V+J+m(h)$
<i>P. socia</i> Mitt. (monoploid)	♀ $K(n)=6=V(X)+3V+J+m(h)$
	♂ $K(n)=6=V(Y)+3V+J+m(h)$
<i>P. socia</i> Mitt. (diploid)	♀ $K(n)=12=2V(X, Y)+6V+2J+2m(h)$
<i>Bartramia pomiformis</i> (L.) Hedw.	♀ $K(n)=8=V(H)+3V+2J+m+m(h)$
<i>B. crispata</i> Shimp.	♀ $K(n)=8=V(H)+3V+2J+m+m(h)$

2) The sex-chromosomes have been found in the four species of *Philonotis*. X and Y are the largest V-shaped heterochromosomes of the male and the female chromosome complements respectively.

3) The intraspecific polyploidy has been found in *Philonotis socia* ($n=6, 12$). The diploid plant is a monoecious having two sets of chromosome complements, each of which is similar in the morphology of the formative elements to that of the male or female gametophyte of a monoploid plant respectively. These evidences are suggestive that the diploid plant may have stemmed from the monoploid one by sporophytic apospory.

キイチゴ属雑種の研究 II

カヂイチゴ♀ × ハチジョウキイチゴ♂について

神 野 太 郎*

Taro JINNO: Studies on the Hybrids in *Rubus* II

R. trifidus Thunb. ♀ × *R. ribisoides* Matsum. ♂

1955 年 5 月 4 日受付

筆者は既にキイチゴ属雑種の研究 I (印刷中)で述べた様に、キイチゴ属植物の種間雑種について研究しており、第 1 報で *Rubus trifidus* Thunb. ♀ × *R. hirsutus* Thunb. ♂ の雑種につき報告したが、引つづき今回は *R. trifidus* Thunb. ♀ × *R. ribisoides* Matsum. ♂ の雑種について報告する。

材料及び方法

この研究は *R. trifidus* を母本とし、*R. ribisoides* を父本として交配して得た雑種第 1 代を対象としたものである。この交配に用いた *R. trifidus* は松山市附近において栽培されていたものであり、*R. ribisoides* は愛媛県三崎半島の海岸に自生していたものである。両親及び両親の栽培場所及び雑種の花粉母細胞における減数分裂の観察のしかたは第 1 報の場合と同じである。

観 察

生育: この雑種に於ては、種子より発芽して本葉が形成される頃より、しばしば生育障害が起つて枯死する個体がある。筆者は 1950 年約 30 個の種子より 2 個体を得た。これらの植物の生育速度は両親植物に比しておそく、又分けつ力も幾分弱い。

形態比較: この F₁ の形態と両親植物の形態とを比較してみると、第 1 表にみる如く両親の何れか一方の形態をしめすものと、両親の中間の形態をしめすものがある。雑種の形態が両親の

うち *R. trifidus* の形態に似るものとしては、1) 花卉の皺: *R. trifidus* は花卉に皺があり、ちぢみの如くなっているが、*R. ribisoides* は皺がなく平滑である。F₁ の花は *R. trifidus* に似て皺がある。2) 花序: *R. trifidus* は聚繖花序をなし、*R. ribisoides* は葉腋に単生花を生じる。この F₁ は *R. trifidus* と同じく聚繖花序をつける。3) 赤色腺毛: *R. trifidus* は莖上及び若い莖に赤色腺毛を生じるが、*R. ribisoides* はいずれの部分にもこれを生じない。この F₁ は *R. trifidus* と同様に莖上及び若い莖に赤色腺毛をもつが、後者の場合における分布の量は *R. trifidus* に比較すると少い。4) 茎の色: *R. trifidus* は茎が紫赤色を帯びているが、*R. ribisoides* の茎は緑色である。この雑種の茎は *R. trifidus* の如く紫赤色を帯びている。雑種の形態が *R. ribisoides* の形態に似るものは大変少くて、綿毛の存在ぐらいである。即ち *R. ribisoides* では葉面上に短毛を有し、葉脈、葉柄及び莖に綿毛を布いているが、*R. trifidus* には綿毛がない。この雑種は *R. ribisoides* の如く葉面、葉脈、葉柄及び莖上に綿毛をもっている。

雑種の形態が両親の中間型をしめすものとしては、1) 葉型: *R. trifidus* は 7 深裂掌状単葉であり、*R. ribisoides* は 3 深裂 2 浅裂単葉であるが、この F₁ の葉は 5 深裂 2 浅裂単葉で両者の中間型をしめす。2) 花の大きさ: *R. trifidus* の花の直径は約 3.4 cm であり、*R. ribisoides* はこれよりも大きくて直径約 4.5 cm の花をつける。F₁ の花は直径約 3.7 cm であるから、*R. trifidus* の方に近い中間型とみられる(第 1 図, A)。3) 其他鋸齒、托葉の形等第 1 表でしめす如く、雑種の

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第1表 *R. trifidus*, *R. ribisoides* 及び F₁-hybrid の形質比較

部 分	植 物		<i>R. trifidus</i>	<i>R. ribisoides</i>	F ₁ -hybrid
	項 目				
花	花 の 直 径		3.4 cm	4.5 cm	3.7 cm
	花 弁 の 皺		有	無	有
	花 序		聚 繖 花 序	単 頂 花 序	聚 繖 花 序
	開 花 の 方 向		上 向	下 向	初期上向, 後期下向
	萼 上 の 腺 毛		有	無	有
	萼 の 展 開		平 開	平 開	平 開
	葉 型		7 深裂掌状単葉	3 深裂2 浅裂単葉	5 深裂2 浅裂単葉
葉	葉面上の短綿毛		無	有	有
	葉柄及び葉脈上の綿毛		無	有	有
	托 葉		長楕円・欠刻あり	狭 長 ・ 全 縁	中間型・欠刻僅かあり
茎	鋸 歯		重 鋸 歯	粗 鋸 歯	中 間 型
	若い茎上の赤色腺毛		有	無	有
	茎 の 色		赤	緑	赤
	茎 上 の 綿 毛		無	有	有
	棘		無	無	無

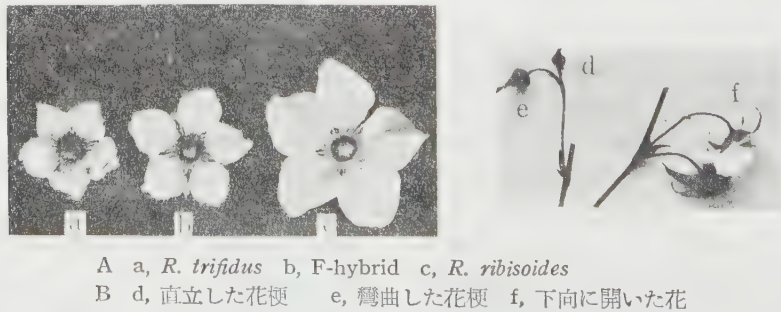
形態は両親の中間型をしめす。

花梗及び花の咲く方向についてみるに、*R. trifidus* は聚繖花序をなし花梗は直立して花を上向きに咲かせるが、*R. ribisoides* は単頂花で花梗は葉腋に下垂して花を下向きに咲かせる。この F₁ は前述した如く聚繖花序をなすが蕾が小さくて直径を

を下向きに咲かせる。即ち初期においては蕾は上向きにつくが、後期においては花梗が斜下方に向き花は下向きに開く（第1図,B）。

稔性：*R. trifidus* 及び *R. ribisoides* の自然における種子の稔性率をみると前者では約68%，後者では約40%内外で何れも種子及び果実を形成

第1図 *R. trifidus*, *R. ribisoides*, F₁-hybrid の花の比較及び F₁-hybrid の花序



5 mm 位迄の初期においては花梗は直立しているが、蕾が膨大し更に花梗も伸びてくるにつれて花梗は漸次弓形に彎曲して先端は斜め下方に向き花

するが、この雑種は不稔性をしめして種子、果実を全く形成しない。

染色体：*R. trifidus* 及び *R. ribisoides* の染色

体はいずれも $2n=14$ で 2 倍体であり、染色体の形態は短棒状で、中部、次中部及び次端部に狭窄をもち V 字形或は J 字形をしているが後者においては一端に付随体をもつ一對の染色体がある。この雑種の染色体数は根端に於て両親と同じく $2n=14$ であることが確められた。個々の染色体は V 字状或は J 字状をしているが、これらの染色体中に付随体をもつ染色体が 1 個存在するのが認められる (第 2 図, a)。

減数分裂: この雑種の花粉母細胞における減数分裂をみるに、デアキネシス期においては 7 個の 2 価染色体が形成される場合が多いが (第 2 図, b), その他一部の染色体が対合せずに 1 価染色体のままで存在する場合もある (第 2 図, c)。減数第 1 分裂中期における染色体の対合状態をみると 7II, 6II+2I, 5II+4I, 4II+6I, 3II+8I 等があらわれる。筆者の調査した 93 個の核板において、これらの現れる頻度をしめたのが第 2 表である。

第 2 図 雑種の体細胞染色体及び花粉母細胞における減数分裂



a 体細胞染色体, b-c 減数分裂 diakinesis 期, a-h 減数第 1 分裂中期, (染色体の黒色は 2 価, 白色は 1 価)
a×1350 b-h×1525

第 2 表 F₁-hybrid 花粉母細胞の第 1 分裂中期における染色体の対合状態

染色体対合 ブロック ナンバー	7II	6II+2I	5II+4I	4II+6I	3II+8I	合 計
1	8	10	5	0	0	23
2	11	15	8	3	1	28
3	14	13	4	1	0	32
合 計	33	38	17	4	1	93
百 分 率	35.5%	40.9%	18.3%	4.3%	1.1%	

この表によると最も多く現れるのは 6II+2I, 即ち 1 価染色体が 2 個現れる場合で全体の 40.9% をしめる。次に多く現れるのは 7II で 35.5% の頻度をしめし、第 3 位が 5II+4I の 18.3% で、これを 7II のあらわれる頻度に比較すると約半分の率である。4II+6I 及び 3II+8I 即ち 1 価染色体が 6 個以上現れる場合は大変少く 4.3% 以下である。このように中期核板における染色体の対合状態をみると 2 価染色体が 7 個, 6 個及び 5 個現れる場合は比較的多くていずれも約 18% 以上をしめすが、2 価染色体がこれ以下の数になると現れる率がきわ立つて少くなる。染色体全部結合して 7 個の 2 価染色体を形成している場合に

においても、互に対合している染色体の間に結合の強調があり、1~2 対のものは互の結合が弱く染色体の一部で対合している状態が見られる。

花粉粒: この雑種の花粉粒をアセトカーミン液で染色して観察すると、細胞内容の充実しているものと内容空虚なものがある。筆者の調査した 1633 個の花粉粒についてみると前者は 714 個 後者は 919 個で、細胞内容の充実している花粉粒の数が内容空虚なものより少くて全体の 45% である。この雑種の花粉粒 142 個についてその大きさの変異をみると大体長径 20μ より 40μ の間にあつて比較的散在して分布する (第 3 表)。しかし員数が最も多く現れるのは 30μ 前後のもの

第 3 表 F₁-hybrid の花粉粒の大きさ

植物	大 小											
	20.1-22p	22.1-24p	24.1-26p	26.1-28p	28.1-30p	30.1-32p	32.1-34p	34.1-36p	36.1-38d	38.1-40p	不定型	合 計
<i>R. trifidus</i>			3	5	31	70	16	14			9	148
<i>R. ribisoides</i>	5	7	12	17	59	45	21	49	4		20	239
F ₁ -hybrid	4	4	17	13	31	11	4	10	15	2	31	142

であり、この点は両親と一致する。花粉粒の不定型の現れる率も両親に比較して高く約 22% をしめす。

論 議

Koidzumi (1913) によると *R. trifidus* 及び *R. ribisoides* は分類学的に別の Subgenus に入り、前者は Subgenus *Anopleobatus* Focke に、後者は Subgenus *Idaeobatus* Focke に属する。この両種とも染色体数は $2n=14$ で 2 倍体であり、*R. ribisoides* には付随体を持つ 1 対の染色体があるが、*R. trifidus* にはこれがない。F₁ 雑種も又同じく染色体数は $2n=14$ の 2 倍体であり、付随体をもつ染色体 1 個が存する。この染色体は父本である *R. ribisoides* より来たものに相違ない。F₁ の形質中には前述した如く両親の何れかの形質に似るものと、両親の中間型をしめすものとがあるが、前者においては母本の *R. trifidus* に似る場合が多く、*R. ribisoides* に似るものとしては葉及び葉における綿毛の存在があげられるに過ぎない。又筆者は既に *R. trifidus* を母本とし *R. hirsutus* を父本として交雑して生じた F₁ の形質について報告したが (印刷中)、それによると無性繁殖力、花の大きさ等雑種強勢をしめすが、*R. trifidus* × *R. ribisoides* より生じたこの F₁ ではこれら形質は雑種強勢を示さず、無性繁殖力は両親よりも弱い。

花梗の方向とこれに関係する花の咲く方向については既に述べた様に、*R. trifidus* は花梗が直立して上向きの花をつけるが、*R. ribisoides* は花梗が下垂して下向きの花をつける。この雑種では初期には花梗が直立しているが、後期には花梗が彎曲して花は下向きに開く、即ち初期には母本である *R. trifidus* の形質をしめし、後期においては父本である *R. ribisoides* の形質をあらわす。これによく似た現象としては池野 (1913) が *Capsicum*

の花梗の遺伝において優劣転換の行われることを観察している。即ち池野は *Capsicum* において花梗が蕾から花、果実を通して上向きの品種と下向きの品種との F₁ が初期は花梗が直立して上向きに花を開き、結実後時期が進むにつれて次第に下向きに変ずることを置いている。そしてこの場合の優劣転換は温度の影響を強く受けるので夏は上向きの花が多く、秋には下向きの花が多くなり、後にははじめから下向きの花をつけると報告されている。筆者の観察したこの雑種では開花期の全期間を通じて、どの時期においても既に開花しているものは下向きに花を咲かせているが、まだ若い蕾の花梗は直立している。即ちどの時期においても下向きの花と直立した蕾が混在していることは、この雑種の花梗の方向の遺伝における優劣転換に *Capsicum* の如く温度等の環境条件が余り関係をしなないものと思われる。

この雑種の減数第 1 分裂中期における染色体の対合状態をみると 6II+2I が最も多く現れて全体の 40.9% をしめ、又 7II 及び 5II+4I の現れる率も比較的高く、前者は 35.5%、後者は 18.3% をしめす。1 価染色体が 6 個以上現れる場合は 4.3% 以下であるから、これの現れる頻度は前 3 者の現れる頻度に比べて大変少い。即ちこの雑種が両親植物の *R. trifidus* 及び *R. ribisoides* より受けたそれぞれのゲノム中 5 対の染色体は親和性が強いが、他の 2 対の染色体は親和性が弱いと考えられる。この雑種は染色体が小さいため、すべての染色体の形態については明確には分らないが前述した如く付随体をもつ染色体が 1 個存在する。これは対をなしていないから親和力の弱い 2 対の染色体のうちの 1 対はこの付随体をもつ染色体を含むものではないかと思われる。この雑種の 14 個の染色体がみな対合して 7II を形成している場合に対をなす染色体間の結合の比較的弱い 1-2 対のものが見られるが、これは前述した親

和力の弱い染色体であると思われる。この雑種は全く不稔であるが、その原因の一つとしては両親植物の遺伝子の相違が考えられるが、染色体組に親和力の弱いものがあることは遺伝子の相違の一

端をしめすものと考えられる。

最後にもたう御指導及び原稿の校閲をして頂いた恩師下斗米教授、並びに植物の同定を御願ひした伊藤博士に感謝の意を表する。

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Résumé

1. This report concerns with the results of the author's observation on the characteristics and the types of pairing of chromosomes in the reduction division of P.M. Cs. in the F_1 hybrid ($2n=14$) which was produced by crossing *R. trifidus* Thunb. ($2n=14$) × *R. ribisoides* Matsum. ($2n=14$).

2. In this hybrid, the characteristics of *R. trifidus* are found in the cyme, the glandular hair, the wrinkles of petals and the color of stems (red-purple), while the characteristic of *R. ribisoides* in the wool on leaves and stems. On the other hand, with regard to some characteristics, e.g. the size of flowers, the shapes of leaves, the serrate and stipule, this hybrid is intermediate of its both parent plants.

3. The peduncle of this hybrid, stands, at first, upright like that of *R. trifidus*, and then it turns downwards like that of *R. ribisoides*. This is a case of dominance change.

4. The types and frequency of pairing of the chromosomes in the metaphase of the first reduction division of P.M. Cs. in this hybrid are as follows: 7II (35.5%), 6II+2I (40.9%), 5II+4I (18.3%), 4II+6I (4.3%) and 3II+8I (1.1%).

5. There are two kinds of pollens in this hybrid; the pollens having contents and the empty pollens. The ratio of their occurrence being 45:55.

本 会 記 事

講 演 要 旨 に つ い て !

日本植物学会第 20 回大会(広島)に一般講演を申込まれた方は下記の要領に従つて講演要旨を送つて下さい。

○ π 切期日 9 月 20 日必着(プログラム編成の都合上期日厳守)

○600 字以内(但図表は含まず)

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投稿される方々のご協力によつて編集も余分な手数がはぶけ、出来上りも可成りスマートになつて来ました。新に投稿される方は次の諸項にご留意して頂きたいと存じます。

1) 表題の書き方、投稿者の所属、文献の引用法等については本年 1~6 月号掲載の各論文を参照。

2) 編集上の都合により原稿並びに図は雑誌発行までお返ししませんから、著者校正のためには手元に同一論文を別に用意しておいて下さい。

3) 著者校正は初校のみですから充分校正の上すぐ編集幹事宛(小石川局区内東京教育大学と明記されたし)返送下さい。再校以後は編集幹事が致します。

4) 投稿先は編集幹事宛直接お送り下さい。

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6) 図、写真の縮少度は幹事一任になつております。

制約された紙面を有効に使うために投稿者各位にご考慮をお願いしたい点は

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ロ) 図について、一般に希望縮少度が大きすぎるようです。ハリカエ可能のように上端だけハリツケして下さい。

ハ) 写真は必要な部分だけにカッティングして下さるか又は必要部分を写真中に明示して下さい。この際、図中の記号はカッティングを考慮してハリツケること (石川茂雄)

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見学 参加 A班 B班 C班 D班 E班 F班 四国方面

不参加

宿舍 要 A級 B級 C級 D級 E級 F級

不要

同宿希望者名

談話会 (御出席の会には○をつけて下さい)

10月12日夜 分類学会 生理学

10月13日夜 形態学及細胞学 生態学会 菌類学会 生理学

(分類学会には植物学会々員は誰でも入会できますから、念のため申添えます)

Studies on a Polyphenolase in *Scopolia japonica* I

On the Oxidation of Catechol*

by Yonezo SUZUKI**

鈴木 与子: スコポリア・ジャポニカの地下茎から得たポリフェノール酵素 I. カテコールの酸化について

Received April 12, 1955

James and co-workers¹⁾ found a polyphenolase in the leaves of *Atropa belladonna*. In the presence of this polyphenolase, catechol is oxidized and when amino acids are present, an intensively coloured compound, red pigment, is formed by the combination of equimolecular proportions of resulted o-quinone with amino acids. The formation of this pigment was also observed by Trautner and Roberts²⁾ by using a polyphenolase from the leaves of *Duboisia myoporoides*. The quinonoid red pigment has an interesting character; it catalyzes the oxidation of amino acids. A cycle of reactions, which, starting with the combination with one further molecule of amino acid, continues with liberation of ammonia and a keto acid was confirmed by James and co-workers, and Trautner and Roberts.

The mechanism as such was also proposed by James³⁾ as a process in the biosynthesis of 1-hyoscyamine in *Atropa belladonna*. The author has an interest in this problem, so as a first step of the research some characters of a polyphenolase from the subterranean stem of *Scopolia japonica* belongs to same *Solanaceae* are investigated.

Material and Method

The subterranean stems of *Scopolia japonica* were washed and peeled. About 30g. of this material was blended with 150 ml. of cold phosphate buffer (M/30). It was filtered with cheese cloth (gauze), centrifuged for 30 min. at 3500-4000 r.p.m. and a white precipitate, which showed positive iodostarch reaction and gave negative colouration with Millon's reagent, was discarded. The supernatant was used as the crude polyphenolase solution. The activity of the enzyme was determined by measuring oxygen uptake in the Warburg apparatus at 25° C and pH 7.3, taking 1.0 ml. of the enzyme solution, 1.0 ml. of phosphate buffer (M/15), 1.0 ml. of inhibitor and 0.5 ml. or 1.5 ml. of distilled water in the main chamber and 0.5 ml. of substrate solution in the sidearm of a flask, the total volume of liquids being made to 4.0 ml.

* This work was announced at the Annual Meeting of the Botanical Society of Japan held in Kyoto on Oct. 27, 1954.

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The center well contained 0.3 ml. of 10% KOH. The gas phase was air.

Results and Discussion

1. Oxidation of Phenols.

The activities of the crude polyphenolase to oxidize various phenols are shown in Fig. 1. The highest O_2 uptake was found in the oxidation of catechol. This

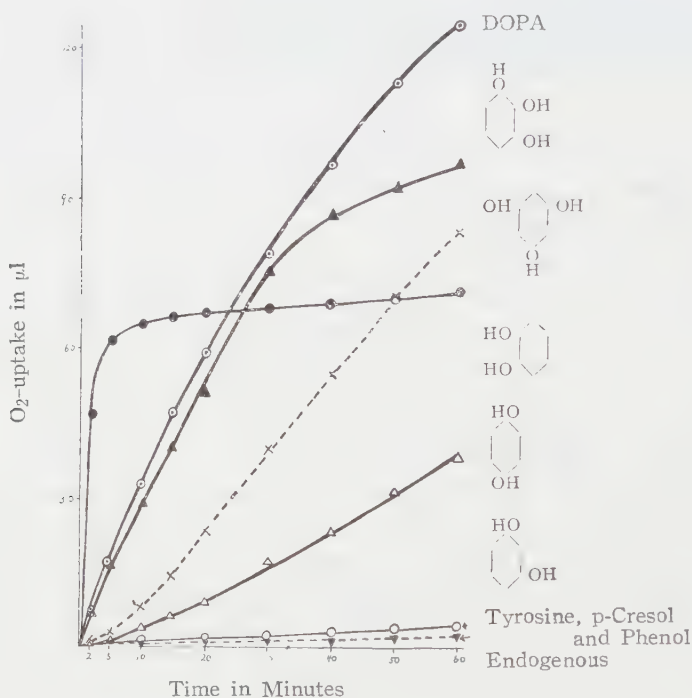


Fig. 1. Oxygen-uptake by various polyphenols catalyzed by the crude polyphenolase.

(The final concentration of substrates was 1.25×10^{-3} M. L-tyrosine was used as suspension.)

initial highest rate, however, slows down quite soon. DL-Dihydroxyphenylalanine (DOPA) and pyrogallol were oxidized readily (DOPA was converted into reddish black substance, melanine?), phloroglucinol and hydroquinone at slower rates, while resorcinol, L-tyrosine, phenol and p-cresol were not oxidized at all. A insoluble form of polyphenolase, which was obtained by treating the leaves of *Atropa belladonna* with acetone, is reported to oxidize p-cresol¹¹. By the use of the present material, however, p-cresol could not be oxidized. It may be said that monophenolase is absent from the present material.

2. Effects of Inhibitions.

The effects of various inhibitors on the oxidation of catechol and phloroglucinol were examined by determining the oxygen uptake. The results are listed in Table

Table I. Effects of Inhibitors on the activity of the crude polyphenolase.

Inhibitors	Final concentration	Inhibition %	
		Catechol (10min.)	Phloroglucinol (60 min)
Cyanide	$0.25 \times 10^{-2}M$	96	81
Azide	$0.25 \times 10^{-1}M$	57	17
Dieca	$0.25 \times 10^{-2}M$	59	95
"	$0.25 \times 10^{-3}M$	35	red
Thiourea	$0.25 \times 10^{-2}M$	0	53
8-oxyquinoline	satu/4	0	red
P-nitrophenol	satu/4	0	red
Iodide	$0.25 \times 10^{-2}M$	0	0
Ferrocyanide	$0.25 \times 10^{-2}M$	6	90
Thiosulfate	$0.25 \times 10^{-2}M$	0	0
Octyl-alcohol	satu/4	0	0
Malachite green (oxalate)	$0.25 \times 10^{-2}M$	12	?
Ferricyanide	$0.25 \times 10^{-2}M$	8	red
Silver nitrate	$0.25 \times 10^{-2}M$	24	red-black
" "	$0.25 \times 10^{-3}M$	5	red-black
Monoiodoacetate	$1.25 \times 10^{-2}M$	14	red
Maleic acid	$0.25 \times 10^{-2}M$	0	red
aa'-Dipyridyl	$0.25 \times 10^{-2}M$	0	red
2. 4. Dinitrophenol	$0.25 \times 10^{-3}M$	0	0

The final concentration of substrate: $1.25 \times 10^{-3}M$.

(oxygen-uptake in catechol only)-(oxygen-uptake in catechol
plus inhibitor)

% Inhibition = 100 × $\frac{\text{(oxygen-uptake in catechol only)}}{\text{(oxygen-uptake in catechol only)}}$

I. The oxidation of these substrates was strongly inhibited by cyanide. Sodium diethyldithiocarbamate (dieca) inhibited strongly the oxidation of phloroglucinol, but it was less effective upon catechol. Thiourea inhibited 50% of the oxidation of phloroglucinol, but it did not inhibit the oxidation of catechol.

In the poisoning effect of azide, an agent also sensitive to copper enzymes was, however, rather weak even at a higher concentration. This might be due to the medium being neutral⁴. 8 Oxyquinoline and p-nitrophenol⁵) were found not to be effective upon the oxidation of catechol. Iodide, ferrocyanide and thiosulphate are known to combine with copper ions in neutral solutions. Among these, ferrocyanide inhibited considerably the oxidation of phloroglucinol, but it inhibited the catechol oxidation quite a little. Iodide and thiosulphate, on the other hand, were not effective upon the oxidation of these substrates.

Ferricysnide, silver nitrate, monoiodacetate and maleic acid are used generally for the purpose of detecting SH groups in enzyme protein, but in this experiment

their effect on the oxidation of catechol was not remarkable.

The results obtained in the present work as well as those of Barron and Singer⁶⁾ may suggest that SH groups are not indispensable for the activity of polyphenoloxidase.

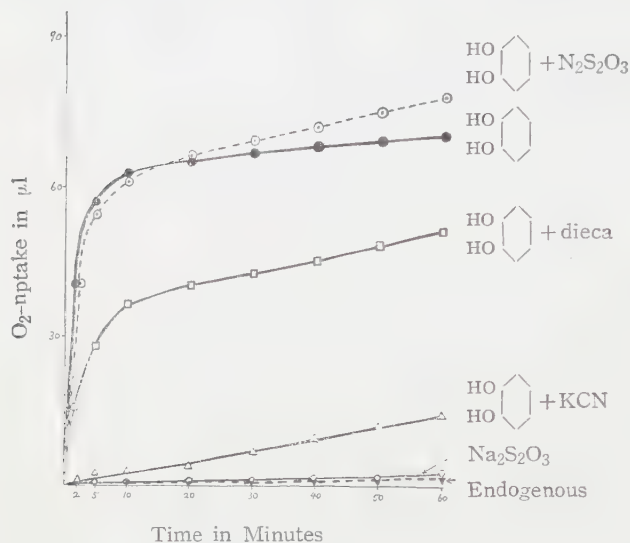


Fig 2. Effects of thiosulphate, dieca and cyanide on the oxidation of catechol.

(The final concentration of catechol was 1.25×10^{-3} M; the final concentration of thiosulphate, dieca and cyanide was 0.25×10^{-2} M)

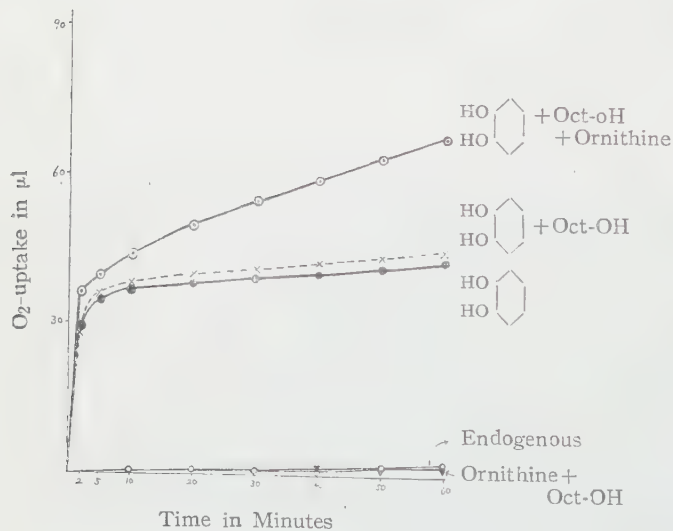


Fig 3. Oxygen-uptake by the crude polyphenolase system containing catechol, ornithine and octyl-alcohol as compared with controls.

(Final concentrations; catechol 1.25×10^{-3} M, octyl-alcohol saturate/4, DL-ornithine-HCL 1.25×10^{-2} M)

As a result of studying various inhibitors it was found that the inhibition of oxygen uptake is accompanied by the failure of the production of the red pigment. Among the inhibitors investigated, thiosulphate did not inhibit the oxygen uptake by catechol, but it hindered the production of the red pigment. The curves of oxygen uptake are shown in Fig. 2.

3. Oxidation of amino acid by the red pigment, in the absence of dehydrogenase.

It is generally said today that the first step in the oxidation of an amino acid is dependent on the dehydrogenases⁷⁾.

As shown in Fig. 3 and Table II, the reaction system constituted of ornithine, catechol, inhibitor of dehydrogenase and crude polyphenolase consumed much more oxygen than that of catechol and crude polyphenolase. These results support the conclusion of James and co-workers¹⁾.

Summary

1. A crude polyphenolase

Table II. Oxidation of ornithine in the presence of red pigment.

Exp. No.	Substrate & others	Oxygen-uptake (μ l) in	
		10 min.	60 min.
I.	catechol	54.0	58.5
	catechol+KCN+ornithine	2.6	10.8
	KCN+ornithine	1.1	4.5
	ornithine	1.5	4.0
II.	catechol	39.6	46.3
	catechol+NaN ₃ +ornithine	31.5	56.5
	NaN ₃ +ornithine	1.9	6.3
	ornithine	2.0	5.5
III.	catechol	35.0	43.5
	catechol+M. G.+ornithine	43.1	61.7
	M. G.+ornithine	1.6	3.7
	ornithine	2.0	4.7
IV.	catechol	34.0	41.8
	catechol+AgNO ₃ +ornithine	29.7	51.5
	AgNO ₃ +ornithine	2.0	5.0
	ornithine	2.0	2.5
V.	catechol	30.7	40.7
	catechol+ferricyanide+ornithine	24.2	54.3
	ferricyanide+ornithine	2.4	5.0
	ornithine	2.4	5.1
VI.	catechol	64.3	72.5
	catechol+Na ₂ S ₂ O ₃ +ornithine	63.4	97.1
	catechol+ornithine	67.7	103.1
	ornithine	2.6	5.0

Final concentrations of substrate and other substances were;

catechol, 0.25×10^{-2} M; DL-ornithine-HCL, 1.25×10^{-2} M; cyanide, 0.25×10^{-2} M azide, 0.25×10^{-1} M; malachite green (oxalate) (M. G.), 0.25×10^{-2} M; AgNO₃, 0.25×10^{-3} M; ferricyanide, 0.25×10^{-2} M; Na₂S₂O₃, 0.25×10^{-2} M.

has been extracted from the subterranean stem of *Scopolia japonica*, into the phosphate buffer.

2. In the presence of this enzyme catechol takes up oxygen at a high rate, DL-dihydroxyphenyl-alanine and pyrogallol are oxidized rapidly, phloroglucinol and hydroquinone rather slowly, while resorcinol, L-tyrosine, phenol and p-cresol are not oxidized at all.

3. Among the inhibitors of the oxidation of catechol, cyanide is very effective, but sodium diethyldithiocarbamate is less effective.

The author wishes to thank Prof. M. Shibata of the Biological Institute and Prof. K. Fukui of the Chemical Institute, Toyama University, for their great interests

in this work.

The material used in the present study was supplied by Prof. T. Nakaoki of the Faculty of Pharmacy, Toyama University, to whom his cordial thanks are due.

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Abnormal Eggs from *Coccophora*, with Special Interests in the Origin of Half Embryos.

by Singo NAKAZAWA*

中沢信午: スギモクの異常卵と半胚の起原について

Received April 30, 1955

There are six different types concerning the course of ovogenesis in Fucaceae, which are in accordance with the systematic arrangement of those⁵⁾. The genus *Coccophora* is separated from *Sargassum*, *Turbinaria*, *Hizikia*, and species of *Cysiothylum*. In the latter a single cell bears eight haploid nuclei in consequence of one hetero- and two successive homoeotypic divisions of a diploid mother nucleus^{1,8,9)}. While in *Coccophora*, seven of the eight nuclei formed in the same way are destined to degenerate with maturation of the oospore remaining one central nucleus¹¹⁾. The former and the latter are also different in the type of cleavage after fertilization. In *Coccophora*, fertilized eggs cleave directly into two daughter cells¹³⁾, while in *Sargassum* etc., when any one of the eight nuclei is fertilized, the other seven degenerate and disappear sooner or later, and the remaining one takes part in development^{1,12)}. In other words, the difference is attributed to the stage of occurrence of the nuclear degeneration, which takes place before maturation in *Coccophora*, but after fertilization in *Sargassum* etc. However, as was studied

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by Getman³⁾ on *Hormosira*, a certain species does not always absolutely stick to a certain type of ovogenesis, but sometimes completely or intermediately appears with another type. The present paper is a report on such a phenomenon observed on *Coccophora*.

During his stay at Asamushi in 1953, the writer observed some abnormal *Coccophora* eggs containing eight nuclei discharged together with normal eggs out of the same receptacle. The phenomenon was repeatedly noticed. The material plant, *Coccophora Langsdorfi* (Turn.) Grey. was collected in the vicinity of the Marine Biological Station at Asamushi, Aomori Ken, on the 12th of April, 1955. It was washed repeatedly with filtered sea-water and then kept in glass vessels over a night, where eggs were liberated on the following day. It is almost impossible that some eggs from another alga such like *Sargassum* have mixed into the present material. In this district, moreover, no another alga of the same family is known to discharge eggs of the same kind in this season. In these respects, the present material is affirmed to be born all from *Coccophora*.

Receptacles were picked from branch and the eggs attached on the surface were scraped off with a glass needle into a Petri dish containing sea water. Among those, one or two out of a hundred were recognized to be eight-nucleate. The ratio of occurrence, however, much varied according to the receptacle. In some cases, over 10 per cent of the whole eggs from a receptacle were eight-nucleate. The abnormal eggs (Fig. 1a, b) so much resemble these of *Sargassum* that they could not be distinguished from the latter. Their course of cleavage was observed after being artificially fertilized together with normal eggs in the same vessel.

The abnormal eggs as well as the normal, whether they are round (Fig. 1a) or a little ovate (Fig. 1b) at the beginning, gradually transformed into more or less ovate form after fertilization. In these abnormal eggs, the first segmentation usually occurs without being accompanied by nuclear division forming a wall at right angles to the major axis, resulting a blunt large cell on one side and a little pointed smaller one on the opposite side (Fig. 1c).

The first segmentation is followed by gradual degeneration of seven nuclei which are contained together in the blunt cell. As the seven out of eight nuclei degenerate, the second and the successive cleavages proceed with occurrence of division of the active nucleus, probably fertilized, contained in the basal or pointed cell (Fig. 1c, d). The division therefore is usually restricted only to the basal half of the egg, where rhizoids are to be formed, and the apical or blunt half remains unicellular containing no nucleus (Fig. 1e, f). This

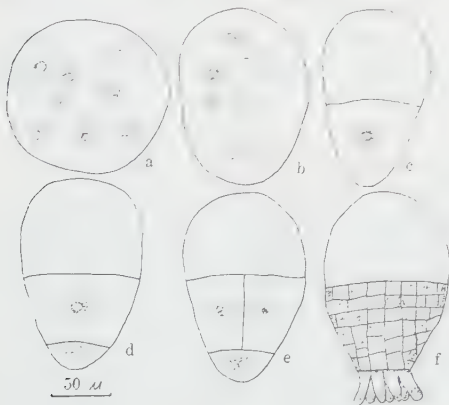


Fig. 1.

tendency that the active nucleus is contained in the basal part seems to verify the opinion presented by Abe²⁾ that the basal pole is differentiated at the entrance point of spermatozoid. The development was traced up to a stage composed of some thirty cells. As the stage proceeds further and the structure becomes more complex, they are unable to be traced distinguished from those originated from the normal eggs. However, some particular embryos with the apical half remaining uncloven (Fig. 1 f) are considered to be a later stage of those abnormal eggs.

Judging from the observations mentioned above, it seems that the ovogenesis and the behavior of nuclei after fertilization of *Coccophora* and those of *Sargassum* etc. are sometimes morphologically continuous. As Inoh⁵⁾ points out in his comprehensive review on Fucaceous algae and now is generally acknowledged, *Coccophora* is taxonomically much more of alliance to *Sargassum* etc. than to *Fucus*, *Pelvetia* etc. within the same family. This seems to confirm the present investigations. Besides, the present studies seem to be able for explaining the origin of the half embryos of *Sargassum* reported by Tahara⁹⁾, Hiroe and Inoh.⁴⁾ That is to say, considering from the writer's observations, it is probable that if the first segmentation independently takes place prior to the occurrence of the nuclear division, as it does sometimes, the fertilized nucleus is naturally to be contained only in one of the daughter cells, the apical or the basal. And as a result, the other cell without diploid nucleus, is destined to remain uncloven till the later stage, the half embryo.

Summary

Coccophora Langsdorfii (Turn.) Grev., a Fucaceous alga, sometimes bears eight-nucleate abnormal eggs (Fig. 1 a, b) together with normal eggs composed of one central nucleus. The abnormal eggs so much resemble those of *Sargassum* as they could not be distinguished from the latter. After fertilization, just like *Sargassum*, seven of the eight nuclei gradually degenerate to disappear with advance of cleavage remaining one large active nucleus, which takes part in the embryo development. The active nucleus, however, is accustomed to be distributed to the basal cell (Fig. 1 c) at the time of the first cleavage which takes place without nuclear division. As a result, the subsequent cleavage is restricted to the basal half, to form a half embryo with a large vacant apical cell (Fig. 1 f).

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Photoperiodic Responses in Japanese Morning Glory, *Pharbitis Nil* CHOIS., a Sensitive Short Day Plant.*

by Shun-ichiro IMAMURA** and Atsushi TAKIMOTO**

今村駿一郎・滝本 敦：短日植物たるアサガオの日長反応

Received May 30, 1955

Japanese morning glory, *Pharbitis Nil* is known as one of the most sensitive short day plants^{1, 3, 6, 9, 11}. Under long day conditions it remains strictly vegetative, and if transferred to short day conditions, it responds promptly to photoperiodic stimulus by initiating flower primordia. The present paper deals in general with the photoperiodic behavior of this plant in comparison with that of other short day plants.

I. Material and Methods. A strain called "Violet" was mainly used in the present study. To secure uniform germination, the seeds were treated with concentrated sulfuric acid for about 30 minutes and washed thoroughly with running water until the next day. They were then sown in boxes in large numbers. As soon as the seedlings appeared, 16 to 25 were planted in boxes in large numbers. As soon as the seedlings appeared, 16 to 25 were planted in boxes measuring 30 cm × 20 cm × 10 cm, filled with garden soil. The plants were grown from the start on a bench in the green house or in the garden under continuous illumination by natural day light supplemented by an incandescent filament lamp of 60 watt from sunset to sunrise. The light intensity at night was ca. 100 foot candles at the leaf surface. When the second or third leaf was fully expanded, the plants were examined for uniformity and only vigorous specimens were used in the experiment; the interior individuals were discarded.

The dark treatment was applied in a dark room arranged in the green house or by covering the plants with wooden boxes of adequate size. After the dark treatment they were returned to the continuously illuminated bench and allowed to grow until collected. The flowering response was determined by careful dissection under a binocular microscope at a magnification of ca. 15.

II. Flowering habit in *Pharbitis Nil* and quantitative measurements concerning flower initiation. In vegetative condition the growing point of *Pharbitis Nil* initiates primordia of foliage leaves in an arrangement of 2:3 parastichies. The axillary buds of each leaf have the same phyllotaxis. When one or two dark treat-

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ments of slightly longer duration than the critical are given, flower primordia are produced at a few nodes near the growing point, but no permanent change occurs in the growing point itself. It continues to form primordia of foliage leaves, in whose axils the differentiating buds produce structures that are entirely vegetative, as was the case with the buds formed prior to the application of short photoperiods. Usually the flower primordia arise in succession, but often one or two vegetative buds appear between two flower primordia.

The first sign of flower differentiation can be readily recognized from the shape of the first two leaf primordia of the bud. As the primordia of the floral leaves appear in closer succession than those of the vegetative leaves, the flower bud has a more or less symmetric form having two relatively equally developed bracts, whereas the primordia of the vegetative leaves are apparently different in their developmental stage and therefore the bud is strikingly asymmetric in shape. Moreover the flower bud is less hairy than the vegetative bud. The first sign of flower initiation can be detected 4 or 5 days after transfer to the photoinductive condition.

If short day induction is strong enough, however, the growing point of the main axis stops to form foliage primordia and begins to form primordia of lanceolate leaves in the axils of which flower primordia arise. Then primordia of bracteal leaves arise, without axillary buds, and at last appear the primordia of the floral leaves of the terminal flower. Thus the growing point is entirely consumed to build up a terminal flower.

Soon after the start of the experiment it was revealed that the photoperiodic response can be more easily observed and quantitatively estimated in the lateral shoot developing after the removal of the main axis than in the main axis itself. The main axis should be removed above a fully expanded young leaf whose axillary bud is allowed to develop. When the short day condition is applied to such a plant, flower primordia appear on the developing lateral shoot. The responses of this lateral shoot are just the same as those of the main axis described above. Three readily determinable numbers, namely those of flowering plants, of plants with terminal flower, and total number of flower primordia initiated can be used in measuring photoperiodic response.

III. Response to various numbers of short days consisting each of an 8-hour light- and a 16-hour dark-period. The plants with two fully expanded leaves were deprived of cotyledons and main axis above the second leaf and transferred to the dark room from 5 o'clock in the afternoon to 9 o'clock next morning. Three lots of 16 plants each received one, two and three such treatments. The remaining lot was left on the continuously illuminated bench and received no dark treatment. Seeds were sown May 20th, 1949 and the experiment was started June 20th. After 18 days the lateral shoot of the second node attained the length of 5cm or more. On examination, the control plants which received no dark treatment had not initiated a single flower primordium. All experimental plants produced flower buds. 16 plants

which received one dark treatment initiated in total 134 flower primordia, and among them 2 plants had terminal flower buds. The plants which received two dark periods had 144 flower primordia and 11 of them produced terminal flowers. The third lot which received 3 dark periods initiated 14 terminal flowers and 148 flower primordia in the total. Comparing the total number of flower buds of the three lots no obvious difference can be found in the intensity of reaction according to the number of treatments. But in the number of plants with terminal flowers the difference is apparent. From the results it may be concluded that *Pharbitis Nil* can be induced to initiate flowers in responding to a single dark period of long duration, as is already known from *Xanthium* and *Chenopodium*^{4, 5, 8, 10}.

On July 8th the apical part of the above mentioned plants was cut off above the first leaf whose axillary bud was allowed to develop. After 14 days the developed axillary shoot was examined for flower initiation. All plants which received dark treatment produced flower primordia. In comparison with the lateral shoot on the second node, the reaction manifested itself in a lesser degree. The difference in reaction according to the number of dark periods given could be seen in the total number of flower primordia and of terminal flower buds. The observation indicates that the dormant bud at the first node had also been photoperiodically induced.

IV. Response to a single dark period of varying duration. Plants sown on the 18th June of 1949 were deprived of the cotyledons and the main axis above the

Table 1. Responses of plants exposed to short day consisting of an 8 hours light period and a 16 hours dark period.

Mode of topping		Number of short days given.			
		0	1	2	3
Top of the plant removed on June 20th. Two cotyledons, the first two leaves remaining.	Number of plants used.	16	16	16	16
	Number of plants with flower primordia	0	16	16	16
	Number of plants with terminal flower bud.	0	2	11	14
Observation on the axillary shoot of the second leaf.	Total number of flower buds.	0	134	144	148
The second leaf and its axillary shoot removed on July 8th.	Number of plants with flower primordia	0	15*	16	16
	Number of plants with terminal flower bud.	0	1	5	13
Observation on the axillary shoot of the first leaf.	Total number of flower buds.	0	79	124	135

* In one plant the axillary bud of the first leaf did not develop.

second leaf. On July 20th 7 lots of 16 plants each were exposed to a single dark period of 9, 10, 11, 12, 16, 20 and 24 hours duration and returned to continuous illumination. The photoperiodic response observed on August 3rd is given in Table 2. Some of the plants exposed to a dark period of shorter than 12 hours' duration

remained vegetative without differentiating any flower primordia. Only one terminal flower was initiated in the lot which received the 20 hour dark period. The total

Table 2. Response to a single dark period of varying duration.

	Duration of the dark period in hours.						
	9	10	11	12	16	20	24
Plants observed	16	16	15	16	16	16	12
Plants with flowers.	2	9	15	15	16	16	12
Total number of flower buds.	4	10	34	40	87	93	60
Number of plants with terminal flower.	0	0	0	0	0	1	0

number of flower primordia was increasing, except for the 24 hour lot, with the lengthening of the dark period. From this experiment a single dark period of 9 hours' duration was revealed to be inductive in some individuals. Therefore, another experiment with a shorter dark period was carried out.

Eight boxes of 16 plants each were divided in two groups. One group received one, the other three dark peroids which varied in duration increasing by one hour from 7 to 10 hours. The experiments started on the 10th of August. Table 3 shows the results obtained after two weeks. The shortest duration of a dark period neces-

Table 3. Photoperiodic response to dark periods of varying duration.

Number of dark periods given.		Duration of the dark period in hours.				
		7	8	9	10	Light control
1	Number of plants used.	16	16	16	16	32
	Number of plants with flowers.	0	0	1	12	0
	Total number of flower buds.	0	0	1	13	0
3	Number of plants used.	16	16	16	16	
	Number of plants with flowers.	0	1	5	16	
	Total number of flower buds.	0	2	10	68	

sary for the least induction was 9 hours in the lots subjected to one dark period and 8 hours in the lots subjected to three such treatments. The number of plants with flower primordia and the number of flowers initiated increased with the increasing number and duration of the dark periods. All plants exposed to three dark periods of 10 hours' duration initiated flowers. The control plants maintained on continuous illumination remained vegetative.

V. Floral initiation caused by a single leaf subjected to a single dark period.

The experiments just reported indicate that *Pharbitis Nil* having developed 2 or 3 leaves can be induced to flowering by a single dark period of sufficient duration.

In order to investigate whether a single leaf when subjected to a single long dark period is also capable to cause floral initiation, another experiment was carried out. 30 days old plants having three fully expanded leaves were used in the experiment on the 11th of August. The fourth leaves were expanding and different in their developmental stages. The plants were deprived of the main axis above the fourth node. In one lot all the leaves, including the cotyledons, and all axillary buds except those on the first node were removed. In other lots the second, the third or the fourth node was left intact and all leaves and buds at the other nodes were removed. The plants were then exposed to a single dark period of 16 hours' duration. As shown in Table 4, all lateral shoots developed on the second and third nodes produced flower primordia. Indications are that the third shoots react more sensitively

Table 4. Responses of plants having a single leaf on various nodes exposed to a single dark period of 16 hours' duration.

Position of nodes bearing donor leaf and receptor buds.	A single dark period of 16 hours duration.				Continuous light (Control).			
	I	II	III	IV	I	II	III	IV
Number of plants with flower primordia. / Number of plants observed.	10/25	23/23	21/21	12/22	0/5	0/5	0/5	0/5
Number of plants with terminal flower.	0	0	4	1	0	0	0	0
Total number of flower primordia.	20	65	159	65	0	0	0	0
Average position-number of the node bearing the first flower.	1.50 ± 0.230	2.26 ± 0.217	2.48 ± 0.181	2.58 ± 0.185	—	—	—	—

to the stimulus than the second, since they produced not only more flower primordia but some of them produced terminal flower buds. This difference in photoperiodic response may be due to the different condition of the leaves receiving dark period treatments or of the buds reacting to the stimulus supplied by the leaves or both. It may be probable that the susceptibility to photoperiodic induction varies with the age of the leaf, as known for *Xanthium* and other plants^{2,10}. Plants with the first leaf reacted less sensitively and plants with the fourth expanding leaf reacted differently according to their stage of development. Some of them remained vegetative, whereas some others produced many flower primordia and even one formed a terminal flower bud.

On the other hand the condition of the reacting bud on different nodes might be also different at the start of the experiment, as revealed by the position of the first flower primordia initiated. The average position of the node bearing the first flower primordium on the first axillary shoot was 1.5, on the second 2.26, on the third 2.48 and on the fourth 2.58. Thus the position of the first flower rises in the axillary shoots of the higher nodes.

As differentiation of the developing bud into a flower bud is determined in an

early stage, the older buds, being already determined as vegetative, cannot be converted to flower primordia. Thus some of the axillary buds at the basis of a lateral shoot remain vegetative. On the higher nodes of the main axis the buds are more advanced in their development at the start of the experiment, therefore more vegetative buds are found on a lateral shoot, which developed upon the removal of the main axis. This could be more clearly shown in the following experiment.

VI. Relation between the position of the first flower primordia and the time elapsed from the removal of the main axis until the start of dark treatment. As the lateral bud begins to develop immediately upon the removal of the main axis, the first flower primordia may arise on the higher nodes of the lateral shoot, if the start of dark treatment is delayed after the topping of the main axis. A single dark treatment of 16 hours' duration was given to the plants, immediately, 2 and 4 days after the removal of the main axis. The experiment started on the 12th of September. The position of the first flower, as indicated by the average position-number of the node bearing the first flower in 32 plants, was 2.44 ± 0.126 , 4.28 ± 0.138 and 6.36 ± 0.192 respectively.

VII. General considerations. Under long day condition or on continuous illumination Japanese morning glory remains vegetative and attains a large size without differentiating any flower primordia, whereas under extreme short day conditions the plant soon stops its vegetative growth converting all growing points into flower primordia and remains dwarfed. Sown in May in the field, it attains a large size and the terminal bud continues to grow producing lateral flower buds and flowering from July to August. Such concomitance of vegetative and reproductive growth could be only realized, when the plant was grown under short day conditions with dark period of slightly longer than the critical duration, for instance of 8.5-9 hours, as prevailing from middle of May to the end of July in middle Japan in consideration of twilight at sunrise and sunset.

The results reported in the present paper show that a plant with a single leaf can initiate flower primordia in response to a short photoperiod. Therefore we have in this plant a more favorable material for investigations of photoperiodic reactions than in any other representatives of short day plants.

As to sensitivity too, morning glory is not inferior to any other plant such as *Xanthium*, soy bean and so forth. But it may be mentioned that the sensitivity varies with internal and external conditions. In a plant with a single leaf, flowering can be induced by a single dark period of 16 hours' duration in most cases but not always. The sensitivity is higher in late spring and autumn. In midsummer, some of the plants do not initiate flower primordia in response to a single dark period. This may be due, in all probability, to the temperature prevailing in this season.

Summary. 1) The critical dark period of Japanese morning glory was found to be ca. 8-9 hours.

2) A plant with a single leaf can be induced to initiate flower primordia by

the application of a single dark period of 16 hours' duration.

3) If the induction is strong enough, the growing point of the shoot is consumed in initiating a terminal flower bud.

4) The number of plants with flowers or terminal flower primordia and the number of flowers initiated can be used in ascertaining quantitative relations in investigations of photoperiodic responses.

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有節サンゴモの解剖分類学的研究 (追報)

サビモドキ (新属新種) の構造と生殖*

瀬 川 宗 吉**

Sokichi SEGAWA: Systematic Anatomy of the Articulated Corallines
(Supplementary Report)

The Structure and Reproduction of *Yamadaia melobesioides* SEGAWA***

1955 年 4 月 12 日受付

筆者は 1940 年から 1949 年¹⁾に涉り、上記の
題目の下に有節サンゴモ各群の代表者を選んで、
その解剖分類学的研究の結果を報告した。

その後、筆者は更に現追報の原稿を用意したの
であるが印刷事情不良のため現在まで発表を延し

ていた。ここに取扱う有節サンゴモは 1934 年伊
豆須崎の海岸に於て採集した極めて特異な種類で
ある (第 1 図)。その外形は *Melobesiae* 即ち無
節サンゴモに極めて類似したものであつて、無節
サンゴモを特に専攻する筆者は当時多数の他の無

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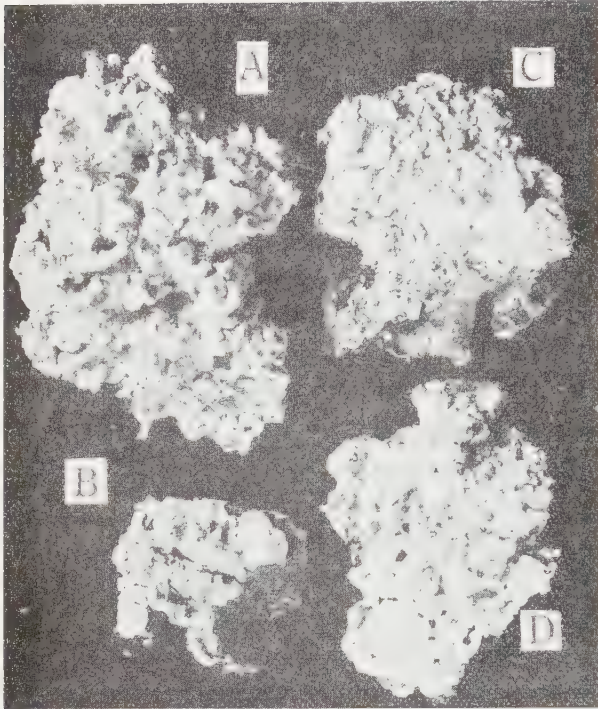
*** *Yamadaia* SEGAWA, gen. nov.

Frons crustaeformis. Pars erecta absens.
Conceptacula prominentia, per crustam sparsa.
Genicula unizonalia, inter crustam et concep-
tacula praesentia.

Type of the genus: *Yamadaia melobe-
sioides* SEGAWA sp. nov.

Characteres iidem ac generis.

Species type: tetrasporic, "Susaki, Izu
Prov., Mar. 27, 1939"; female, "Susaki, Izu
Prov., Apr. 1941"; male, "Susaki, Izu Prov.,
Mar. 27, 1939" (in the Herbarium of Mitsui
Institute of Marine Biology).

Fig. 1. Matereals. ($\times 4/5$)

- A. ♂ individual. "Susaki, Izu Prov., Mar. 27, 1939"
 B. ♀ individual. "Susaki, Izu Prov., Apr. 1941"
 C. ⊕ individual. "Susaki, Izu Prov., Mar. 27, 1939"
 D. ♂ individual. "Susaki, Izu Prov., Apr. 1941"

節サンゴモと共に採集し固定し研究を始めた。体の大部分が無節サンゴモ同様皮殻を形成し基質に着生して居るに拘わらず、その生殖葉はその皮殻の中に形成されず必ず外部に超出し、然も皮殻部との境は必ず可動的の膝節によつて区別せられる。発見当時経験の浅かつた筆者はこの特異なサンゴモを分類上如何なる位置に置くべきか甚だ迷つたのである。然しその後無節サンゴモの研究も進み且又有節サンゴモの解剖分割学的研究の進むに従い、次第にその点が明らかになつて来た。加うるに発見当時採集出来なかつた♂個体はまず1939年、♀個体は1941年漸く採集出来、ここに3種の個体も揃い愈々その位置も明らかとなつて来た。そこで此のサンゴモの爲新属を設定し、且解剖的の観察を行い、他の有節サンゴモと比較し、その系統に就て論じようと思ふ。属名 *Yamadaia* は本研究の爲終始懇篤なる御指導を下され、校閲の勞を執られた恩師山田幸男教授に献じたもので

あり、和名サビモドキはその外形が余りにも無節サンゴモの某種に類似せるに基いたものである。

体 の 構 造

サビモドキは他の有節サンゴモと異り、体節・膝節交互する直立部を全然有せず、甚しく発達した皮殻部を有するものである。もとより他の有節サンゴモも孢子発生の初め皮殻部をまず作りその上に直立部を作るのであるが、皮殻部の発達直立部に比して著しく悪く、その為眼に触れないのが普通である。従つて或種の如きは皮殻部の発見に困難を感じる如き場合もあるのである。本種の皮殻部の放射断面を作つて見るに大体 *Melobesia* のものに於ける如く *Hypothallium*, *Perithallium* 及び *Epidermis* の3層を区別する事が出来る。 *Hypothallium* は円柱状の細胞から成り、その細胞糸は体の下部に於いて水平に走り漸次彎曲し直立して *Perithallium* となる。体の最下部に於いては細胞糸が少しく下方に彎曲するのが普通である。 *Perithallium* の細胞は *Hypothallium* に於けるよりも短

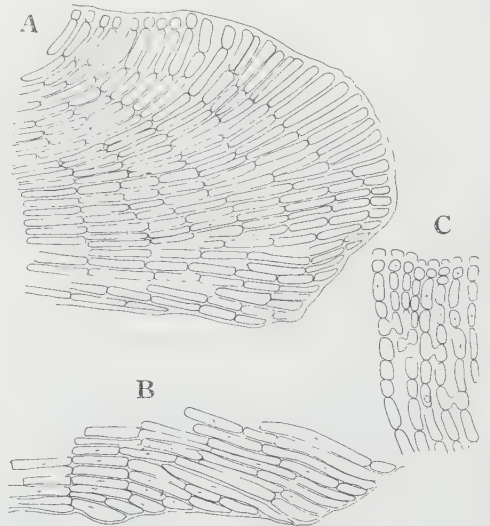


Fig. 2. Longitudinal section of the crust. ($\times 250$)
 A. Margin.
 B. Hypothallium.
 C. Perithallium.

く幅も狭い。是等両層に於ける細胞の横の配列は整然たるものではなく相当乱れて居る。然し細胞糸が互に交錯する事は見られない。実質は両層に於いて正方形に近い細胞の1層から成り、体の古い部分は時に2層以上の事がある。Perithalliumの最上層の細胞に冠せられる。然し生長線には表皮細胞は現れない。又 Hypothallium の下層にも現れない。生長線には内容の多い長い分裂細胞

となつて生殖窠を有する1体節 (Segment) を形成するのである。この際の膝節細胞の核の動向はあまりよく追究されなかつたが、概して云えば膝節細胞の伸長し初めにはずつと下位に存在し、次いで体節細胞の1層 (以後の分裂層となる) を分離した当時は核の位置が極めて不同である。次に膝節が完成すれば核の形は長形となり中央部より上位に位置するに到る。



Fig. 3. Development of the node. ($\times 250$)

の1群が占めている。

膝節 (第3図) 膝節は生殖窠の基部に限つて形成される事は前述の通りであつて、生殖窠は皮殻部の表面に膝節の柄を持つて超出して居る。甚だ長い細胞の1層から成り、両端が Extragenicular portion となつて居る事は他の大多数の有節サンゴモの場合と同様である。

膝節の発生はまづ皮殻部の Perithallium の最上部に相当する細胞の1群が分裂組織に変わる事に初まる。或る一定の長さに達すればその各々の先端には1個の短細胞が分裂される。この時期には未だ Extragenicular portion の分化が認められない。この分裂された細胞がその後の分裂細胞

尙膝節の周囲は後に幾分消失し、その膝節細胞によつて形成せられた体節の皮層の部分は皮殻部と直接の連絡を絶たれる。

相異る細胞列間の横の連絡 Hypothallium, Perithallium 及生殖窠を有する体節の髄層皮層を越じ、連絡溝を随所に見出す事が出来る。第二次連絡点は見出すことは出来なかつた。この事は第二次連絡点を有するヒノイソヤリ、ヒメカキノテ、Lithothrix 等を含む群とは類縁の薄いつつの証拠と思われる。尙前述の分裂層の表面に表皮細胞を持つていない事も又同様一つの論拠となる。

生 殖 窠 (第 4, 5, 6 図)

生殖窠は体の上面に散在し、膝節に依つて皮殻部と境する。云わば 1 生殖窠そのものが 1 体節を形成して居る。尙附言する事は sterile の体節と云うものはこの種類に於いては今まで観察されなかつた事である。何れの生殖窠に於いてもその形成の初期に将来窠底となるべき中央部の成長の停止がおこり、その縁部が成長を続けて窠蓋が形成されて行き、窠腔の中央直上に開口を残して完成する。

サビモドキの生殖窠は側生であろうか、頂生であろうか、又はその何れに近いものであろうか。サビモドキの 1 体節より成る直立部は他の有節サンゴモに於ける直立部が最も退化した場合と見倣

即ち生殖部位の細胞が髓より起る事からもこの事は云われ得ると思う。

雌性器官 (第 4 図) 完成した生殖窠は頂端が鈍円で長倒卵形である。始め成長の停止した窠の中には色素で内容の濃染する原始細胞が並列して見られる。窠底が窠蓋に依つて覆われるに到れば各々の細胞は 1 組の Procarp をつくる。Procarp は 1 個の基部細胞に対して 1 個の Carpogonial branch と 1 又は 2 個の中性細胞が組となつて居る。受精後窠底に癒合細胞が出来る。癒合細胞は薄くて造胞糸が発達する頃になつても尙断面に於いて処々切れて居る場合が多い。造胞糸は癒合細胞の縁部から発達して来る。完成した雌窠の窠腔はもとの空隙よりも拡張の結果広くなり、形は卵形に近い。



Fig. 4. Female conceptacle.

A. Young conceptacle. ($\times 117$) B. Procarps. ($\times 167$) C. Conceptacle with procarps. ($\times 117$) D. Conceptacle with gonimoblast filament. ($\times 117$)

す事が出来る。即ち中間の sterile な体節は発達せずに生殖窠の発達する体節のみ残存したものである。従つて膝節の上に分裂して出来た頂端分裂層の相繼ぐ分裂に依つて形成された生殖窠は頂生と云わるべきであらう。解剖的に見て窠底の細胞

雄性器官 (第 5 図) 完成した雄性器官を有する体節は他のものに比し、その先端が尖つて居る。外観は紡錘形を呈する。窠腔の形状は断面に於いて半円形で弧の部分が窠底にあたる。窠溝は甚だ長い。窠底は屢々 2 つの急彎曲を認める事が出来



Fig. 5. Male conceptacle. ($\times 117$)
A. Young conceptacle, B. Mature conceptacle.

その彎曲に依つて寛底、寛蓋とを区別する事が出来る。精子の母細胞は寛蓋の基部一帯に生ずる。即ち寛底と認められる部のみならず寛蓋の部にも生ずる。精子の母細胞は密に相並びその各々は長形な未熟精子細胞を着生して居る。精子は長尾状附屬物を有するらしく思われるが此の点は副瞭に出来なかつた。

四分孢子囊窠 (第 6 図)

成熟した孢子囊窠を有する体節は雌性のものと似て居てその外貌は長楕卵形に近い。窠腔は円形で割合に長い窠溝を持つて居る。窠腔は始め小形であるが後に拡張を行い大形となる。将来寛底となるべく成長を停止した筈の中には初め分裂細胞と同様内容の豊富な一群の細胞を認める事が出来る。又成熟した生殖窠の窠底には並立した四分孢子囊が認められる。そしてこれら両者の中間の段階にあるものには往々 Paraphyses を認める事が出来る。

要 結

以上の結果を要約すれば、

- (1) 本種の皮殻部は甚だしく発達し、此に反して直立部は単にその表面に散在せる生殖窠を有する 1 体節に依つて代表せられる。
- (2) 皮殻部は Hypothallium, Perithallium 及 Epidermis の 3 部から成る。
- (3) 皮殻部の周辺は長形の分裂細胞より成り表皮細胞によつて覆われて居ない。
- (4) 膝部は皮殻部と生殖窠の間にのみ形成せられ、1 層細胞より成る。
- (5) 隣接細胞列に属する細胞相互の連絡は連絡溝を以つてする。
- (6) 膝節細胞相互の連絡は形態的には認められない。
- (7) 各種の生殖窠は唯一つの体節に作られ頂生の型に属する。
- (8) 各種生殖窠の窠蓋は窠底周囲の部の特別の成長に由來す。



Fig. 6. Tetrasporangial conceptacle. ($\times 117$)
A. Young conceptacle. B. Mature conceptacle.

(9) 雌性の生殖窠は長卵形，窠腔の断面は卵形である。

(10) Procarp は 1 の基部細胞に対し，1 の Carpogonial branch と 1 又は 2 の中性細胞より成るのが普通である。

(11) 造胞糸は薄い癒合細胞の周辺からだけ生ずる。

(12) 雄性生殖窠は先端尖り，長紡錘形，窠腔は半円形，窠溝は長い。

(13) 精子母細胞は窠底のみならず窠壁にも生ずる。

(14) 四分孢子囊窠は長倒卵形，窠腔の断面は円形である。

(15) 四分孢子囊は窠底のみに生じ，Paraphyses は僅かに生ずる。

本種が狭義の *Amphiroa* 並に *Lithothrix* とはその類縁が遙に遠い事は論の無い処である。又 *Metagoniolithon* も遙かに遠いと思われる。*Bossea*, *Joculator* と段々類縁が近くはなるがまだまだ共通点は少い。*Calliarthron*, *Arthrocardia*, *Duthiea*, *Cheilosporum* も少しく遠い感がある。結局 *Corallina* か *Jania* に近い事がわかる。此

処に注意すべきは BOERGESSEN (1915)²⁾ が西印度から報ずる処の *Jania pumila* であつて同氏の観察した *Turbinaria* の体上に生育して居た同植物は屢々皮殻部の上に 1 体節を生じ，そのものが生殖窠を有するものである。“The erect filaments are of variable size and development. Some of them consist of a single joint which on its top bears a conceptacle, others are longer, a few times dichotomously divided, ending in conceptacles, if they are not throughout vegetative.” と氏は記述している。此の植物を考慮に入れる時はサビモドキ必ずしも独り飛び離れて系統上の位置を取るものとは思われぬ。 *Jania* と或聯関を有する事が予想出来る。然し乍ら 1 体節以上に直立部が発達せぬ事及びこの体節は例外なくその先端に生殖窠を着ける事は 1 独立属としての性質として充分であると認めるのである。尙此処に注意すべきは，この植物の解剖的性質が *Jania* よりむしろ *Corallina* に近いと考えられる節のある事である。もし *Corallina* と *Jania* と相分離すべきものであるとすればむしろ *Corallina* に近く置かるべきものであろう。もし

又 *Corallina* と *Jania* がある学者が考えた如く
非常に相近い群であるとすれば勿論是等 3 者が 1

つの群となり他の群よりも高位に置かるべきもの
であろう。

Summary

Yamadaia, a new genus proposed in the present paper, is a very curious articulated coralline. It was discovered by the writer on the coast of Izu Province.

The erect part of the species is reduced to one joint only and the crust is so vigorously developed that, at first sight, it is mistaken for a non-articulated coralline.

The node is unizonal and formed only between the crust and the joint. Thus, the erect part is represented only by a single joint bearing a conceptacle. The conceptacle-bearing joints are scattered on the surface of the crust.

As in crustaceous Corallinaceae, the crust is made up of three layers: the epidermis, the perithallium and the hypothallium. The marginal meristem of the crust is composed of one layer of the long oblong cells and is not covered with an epidermal layer. Only transverse canals are found between cells belonging to different filaments and no morphological connexions are found among cells of the node. The conceptacle has an opening on its top and belongs to the terminal type. The roof of the conceptacle is built up of a special growth of the tissue around the bottom of the young one. The female conceptacle is long obovate in external form, and its cavity is ovate in section. The procarp is composed of one basal cell, one carpogonial branch and one or two sterile cells. The gonimoblast filaments are produced only from the margin of the thin fusion cell. The male conceptacle is long spindle-shaped in outline, and the beak is so long that the conceptacular canal is very long. The spermatangial mother cells are produced not only from the bottom but also from the lateral wall of the cavity. The tetrasporangial conceptacle is long obovate as in the female one, and the cavity is circular in section. The sporangia issue only from the bottom, and are surrounded by paraphyses few in number, in their young stage.

The above mentioned respects show that the present genus has a close relation to *Jania* on the one hand, and to *Corallina* on the other hand. The anatomical characters rather suggest an intimate relation to *Corallina*.

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キンボウゲ科の細胞学的研究 III

ヒエンソウ属、レイジンソウ属及びトリカブト属における数種の核型

栗 田 正 秀*

Masahide KURITA: Cytological Studies in Ranunculaceae III

The Karyotypes of Several Species in *Delphinium*, *Lycotomum* and *Aconitum*

1955 年 5 月 30 日受付

キンボウゲ科の次の 3 属、すなわちヒエンソウ属 (*Delphinium*)、レイジンソウ属 (*Lycotomum*) 及びトリカブト属 (*Aconitum*) は分類学上近縁な位置におかれている。ヒエンソウ属の染色体は Tjebbes¹⁰⁾ によると Overton (1893), Osterwalder (1868) 及び Bönicke (1911) によつて観察されているようであるが、その後 Langlet,^{3,4)} Tjebbes,¹⁰⁾ Lewitsky,⁶⁾ Lawrence⁵⁾ 等によつて研究された。レイジンソウ属の染色体は Lewitsky,⁶⁾ Schafer 及 La Cour⁹⁾ 等により、トリカブト属のそれは Langlet,^{3,4)} Lewitsky,⁶⁾ Afify,^{1,2)} 酒井⁸⁾, Longacre⁷⁾ 等によつて研究された。これらの諸研究者の報告のうちで、核型について詳しく記述しているのは Lewitsky,⁶⁾ Schafer 及 La Cour⁹⁾ 及び Lawrence⁵⁾ のそれである。

筆者も上記 3 属に属するさらに多数の種において核型分析をおこない、3 属間の関係を明らかにしたい目的で研究をおこなっているが、本報告ではヒエンソウ属 1 種、レイジンソウ属 3 種、トリカブト属 1 種 1 変種の核型を報告する。方法は前報告でのべたのと同じである。

観 察

1. ヒエンソウ *Delphinium Ajacis* L. (松山市内栽培)

本種の染色体数は Tjebbes¹⁰⁾ によると Osterwalder (1898) 及び Bönicke (1911) 等によつて $n=12$ と報告されているようであるが、Tjebbes¹⁰⁾ により $n=8$, Langlet,^{3,4)} Lewitsky⁶⁾ 及び La-

wrence⁵⁾ により $2n=16$ と報告された。

筆者も本種の染色体数を $2n=16$ と決定した。これら 16 個の染色体はその形及び大きさから次のように 4 種類に区別できる (第 1 図)、すなわち 1) 最大の 1 対の染色体 (同図, a)。この着糸点は中部にあり、1 腕末端に微小な付随体をもつ。2) 前者よりきわめてわずかに短い、中部着



Fig. 1. Somatic chromosomes of *Delphinium Ajacis*. $\times 947$

糸の 1 対 (同図, b)。3) 前 2 者に比べ明らかに短い 5 対 (同図, c-g)。いずれも次端部着糸であつて、各対はきわめてわずかに長さに差がある。4) 最小の 1 対 (同図, h)。次端部着糸で、長さは 3) のうちの最小のもの (同図, g) よりやや明らかに短い。

上述から本種の核型は次の式で示せる。

$$K(2n) = 16 = 2t \text{ Am} + 2\text{Bm} + 10\text{Cst} + 2\text{Dst}$$

これまでにヒエンソウの核型をもつとも詳細に分析したのは Lewitsky⁶⁾ であろう。氏の結果と上にのべた筆者の結果とを比較するとほぼ一致するが、筆者は大形の中部着糸の染色体 1 対におい

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て付随体をみとめたが Lewitsky はそれを観察していない点で明らかにことなる。

2. イブキレイジソウ *Lycotemon chrysopilum* Nakai (滋賀県伊吹山産)

本種の根端細胞で 16 個の染色体をみとめた。これらはその形及び大きさから次のように 5 種類に区別することができる (第 2 図)。すなわち 1) 最大の 1 対の染色体 (同図, a)。この着糸点中部にある。2) 前者よりわずかに短い次端部着糸の 1 対 (同図, b)。5 核板におけるこの染色体 10 個の測定結果によると長腕 : 短腕 = 100 : 37 であった。3) 前者より明らかに短い次端部着糸の 1 対 (同図, c)。短腕に小さい付随体をもつが、この付随体は常に容易にみとめられる。前と同様に 10 染色体の測定結果によると長腕 : 短腕 = 100 : 42 である。4) 前者より更に 1 端よりに着糸点をもつ 4 対 (同図, d-g)。各対はきわめてわずかに長さに差がみられる。このうちの最大染色体 (同図, d) は 3) の染色体よりわずかに長くみとめられる核板が多かつた。5) 最小の 1 対 (同図, h)。前記 4) のうちの最小のもの (同図, g) より明らかに短かく、次端部着糸点をもつ。

本種の核型は次のように示せる。

$$K(2n) = 16 = 2A_m + 2B_{st} + 2t C_{st} + 8D_{st} + 2E_{st}$$

3. レイジソウ *L. Loczyanum* Nakai (愛媛県石槌山産)

本種の体細胞染色体は 16 個である。核型は前述のイブキレイジソウのそれとよく似ているが、ただ付随体について次の点でことなる。すなわちレイジソウ (第 3 図) は 1. a-染色体に微小な付随体をもつこと。2. c-染色体に付随体のみとめられないこと。3. d-g-染色体のうちの 1 対 (同図, g) に付随体があること。4. h-染色体に付随体があることの 4 点でイブキレイジソウとはことなっている。なおレイジソウではその h-染色体の付随体は他の 2 つの付随体より常に容易に観察することができる。

本種の核型は次のようにあらわせる。

$$K(2n) = 16 = 2t A_m + 2B_{st} + 2C_{st} + 6D_{st} + 2t D_{st}^* + 2t E_{st}$$

4. アヅマレイジソウ *L. pterocaulum* Nakai (長野県戸隠山産)

本種の染色体数は $2n = 16$ である。核型 (第 4



Figs. 2—4. Somatic chromosomes of *Lycotemon*. 2, *L. chrysopilum* 3, *L. Loczyanum* 4, *L. pterocaulum* $\times 947$

図) はイブキレイジソウのそれとよく似るが、付随体について次の 2 点でことなっている。すなわち 1. イブキレイジソウの a-染色体には付随体はみられなかつたが、アヅマレイジソウの a-染色体には大きい付随体が存在する。2. イブキレイジソウの h-染色体には付随体はみられなかつたがアヅマレイジソウの h-染色体には付随体が存在する。本種の a-染色体の付随体は前記 2 種のいずれの付随体よりも大きく、常に容易に観察することができる。

核型は次のようにあらわせる。

$$K(2n) = 16 = 2t A_m + 2B_{st} + 2t C_{st} + 8D_{st} + 2t E_{st}$$

上に述べたレイジンソウ属 3 種の核型は付随体を考慮しなければ互に非常によく似ており、かついずれも Lewitsky⁶⁾ によつて研究され、付随体のみとめられていない *Aconitum Lycoctonum* の核型と一致する。

5. ホソバトリカブト *Aconitum senanense* Nakai (長野県三伏峠産)

根端細胞の染色体は 32 個である。これらはその形と大きさから次のように 5 種類に大別することができる (第 5 図)。すなわち 1) 中部着糸の大形染色体 2 対 (同図, a b)。このうち 1 対では 1 腕に小さい付随体がみとめられる。2) 次端



Figs. 5-6. Somatic chromosomes of *Aconitum*. 5, *A. senanense* 6, *A. japonicum* var. *montanum* × 947

部着糸の大形染色体 2 対 (同図, c d)。このうち 1 対の着糸点是他対のそれよりわずかに内端によつている。3) 前染色体に比べて明らかに短い染色体 2 対 (同図, e f)。いずれもその着糸点は次端部にある。4) 着糸点が前者のそれより更に 1 端によつたところにある短い染色体 8 対 (同図, g-n)。このうち 2 対 (同図, m n) ではその短腕に付随体が存在する。5) 前者のうちの最小のもの (同図, l) より明らかに短い染色体 2 対 (同図, o p)。各対の着糸点は次端部にある。

本種の核型は次の式で示せる。

$$K(2n)=32=2^t A_1^m+2A_2^m+2B_1^{st}+2B_2^{st}+$$

$$4C^{st}+12D_1^{st}+4^t D_2^{st}+4E^{st}$$

6. ヤマトリカブト *A. japonicum* Thunb. var. *montanum* Nakai (愛媛県皿ヶ嶺産)

本種の染色体数は $2n=32$ である。核型は前記ホソバトリカブトのそれとよく似るが次の点でことなる (第 6 図)。すなわちホソバトリカブトでは 4) で述べた 8 対の染色体のうち 2 対に付随体がみとめられたが、ヤマトリカブトではその 8 対に相当する染色体 (同図, g-n) のうちの 1 対 (同図, n) に付随体がみられる。

核型は次の式にあらわせる。

$$K(2n)=32=2^t A_1^m+2A_2^m+2B_1^{st}+2B_2^{st}+$$

$$4C^{st}+14D_1^{st}+2^t D_2^{st}+4E^{st}$$

考 察

ここに報告したヒエンソウ属、レイジンソウ属及びトリカブト属植物の核型を付随体を考慮せずに比較してみよう。付随体を考えなければ既述のようにレイジンソウ属の 3 種はいずれもよく似た核型を示し、トリカブト属の 1 種 1 変種もそうであるから、前者の代表としてイブクレイジンソウ (第 2 図) を、後者のそれとしてホソバトリカブト (第 5 図) をもちいることにする。

ヒエンソウの核型とイブクレイジンソウのそれとは次の点で異なる (第 1—2 図参照)。すなわち半数染色体組でいえばイブクレイジンソウには長腕に対して割合長い短腕をもつ次端部着糸の染色体 2 個 (第 2 図, b c) があるが、このような染色体はヒエンソウにはみられない。これに反しヒエンソウはイブクレイジンソウではみられない第 1 図 b で示され中部着糸の大形染色体 1 個をもち、さらに同図 c-g で示された染色体のうちの何れか 1 個をイブクレイジンソウよりより多くもっている。

ホソバトリカブトの核型とイブクレイジンソウのそれとを比較すると (第 2 図及び第 5 図参照)、イブクレイジンソウの a-染色体はホソバトリカブトの a-及び b-染色体に、b は c 及び d に、c は e 及び f に、h は o 及び p にそれぞれ似ており、イブクレイジンソウの d-g で示された 8 染色体の重複がホソバトリカブトの g-n で示

された 16 染色体に相当するものとみられる。すなわちホソバトリカブトの染色体構成はイブキレイジンソウの半数染色体組によく似た組 4 つより成り立っているものとみることができ、したがって染色体形態上ホソバトリカブトは同質 4 倍体であると推察される。しかし、さながらにホソバトリカブトの c- 及び d-染色体は同一形態ではなく、やや異なる位置にあるのであるから、染色体の位置による異なる倍染色体の変化がおこつておるのではなからうか。

上述から明らかなように基本核型からみてレイジンソウ属植物はヒュンソウよりもトリカブト属植物によりよく似ているといえる。

御懇切な指導を賜つた下斗米教授に厚く御礼申し上げます。慶応大学水野忠敦教授からはトリカブト属の染色体について種々御教示をいただき、大阪大学南校田村道夫氏からは分類学上の御援助と貴重な材料とを恵与された。ここに記して両氏に深く感謝する。

Summary

1. Karyotype analysis was carried out on five species and one variety. Their karyotype formulae are represented as follows:

Delphinium Ajacis $K(2n)=16=2^t A^m+2B^m+10C^{st}+2D^{st}$

Lycotconum chrysopilum $K(2n)=16=2A^m+2B^{st}+2^t C^{st}+8D^{st}+2E^{st}$

L. Loczyanum $K(2n)=16=2^t A^m+2B^{st}+2C^{st}+6D_1^{st}+2^t D_2^{st}+2^t E^{st}$

L. pterocaulis $K(2n)=16=2^t A^m+2B^{st}+2^t C^{st}+8D^{st}+2^t E^{st}$

Aconitum senanense $K(2n)=32=2^t A_1^m+2A_2^m+2B_1^{st}+2B_2^{st}+4C^{st}+12D_1^{st}+4^t D_2^{st}+4E^{st}$

A. japonicum var. *montanum* $K(2n)=32=2^t A_1^m+2A_2^m+2B_1^{st}+2B_2^{st}+4C^{st}+14D_1^{st}+2^t D_2^{st}+4E^{st}$

2. The haploid chromosome set of *Lycotconum*-species bears a much stronger resemblance to the basic set of *Aconitum*-species than to the haploid set of *Delphinium Ajacis*.

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本会会員 藤岡光長氏は昭和 30 年 7 月 16 日死去されました。

ここに報告し謹んで哀悼の意を表します。

日本植物学会

Cytogenetics of the Indian Jasmines I Morphological and Taxonomical*

by V. S. RAMAN**

Received May 7, 1955

I. Introduction: The family *Oleaceae* comprises of about 20 genera and over 500 species distributed in the tropical and temperate regions of the World (Bailey 1949). Engler and Prantl (1897) cite about 160 species of *Jasminum* in the tropical and sub-tropical regions of Asia, Africa, Australia, and America and over 40 in India. Gamble (1936) describes 20 species from Madras Presidency.

The commonly cultivated jasminums are, *J. sambac* Ait., *J. auriculatum* Vahl., *J. flexile* Vahl. and *J. grandiflorum* Linn. Bor and Raizada (1946) and Krishnaswamy and Raman (1948) have given taxonomic descriptions of some species of the genus. Nalini Nirodi (1959) recorded an abnormal jasmine in which the stamens have arisen by the transformation of petals. This paper gives a short description of four varieties and forms of *J. sambac* and also the differences in leaf and flower characteristics between the wild and cultivated forms of three species (Tables I and II).

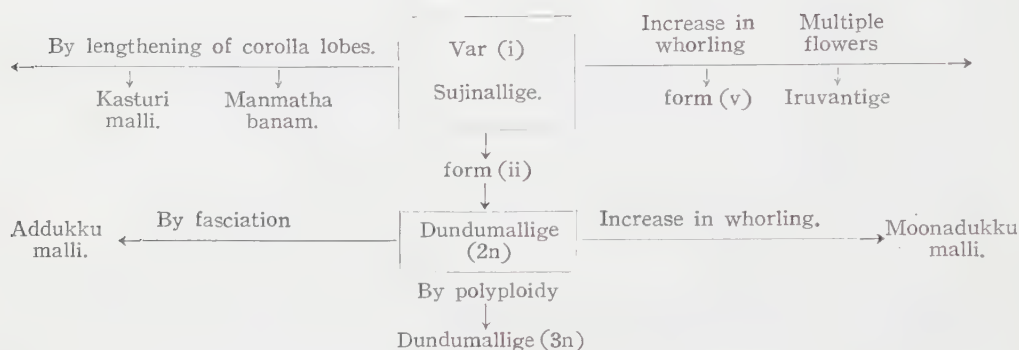
II. Materials: The species and varieties treated in this study were collected from Madras State.

III. Observations and Discussion: (a) The seedling Jasminums: Fruit set is common in all the wild species. Chances are remote for the cultivated ones to bear fruits as the flowers even in their bud stage are plucked off. During this operation, the epipetalous stamens naturally go off with the buds. As these cultivated ones have spread through clonal propagation, all collections of a species or variety show similarity in their morphological characteristics. Selfed flowers do not set fruits, but do so when left for open pollination. Seedlings were raised from plants listed in Tables I and II and all these were true to the chromosome number of their parents. However, variation in the size and shape of leaves and leaflets was observed. (b) Types of floral abnormality in *J. sambac* varieties: The simple type of flower has one whorl of either elliptic or ovate corolla lobes with a single corolla tube. The first type of origin is by simple fission of the corolla lobes, increase in the number of whorls without affecting the corolla tube and the two epipetalous anthers. The corolla lobes remain single or two whorled but the anthers become petaloid Type II. In type III, two or three different flowers each with its own corolla tube

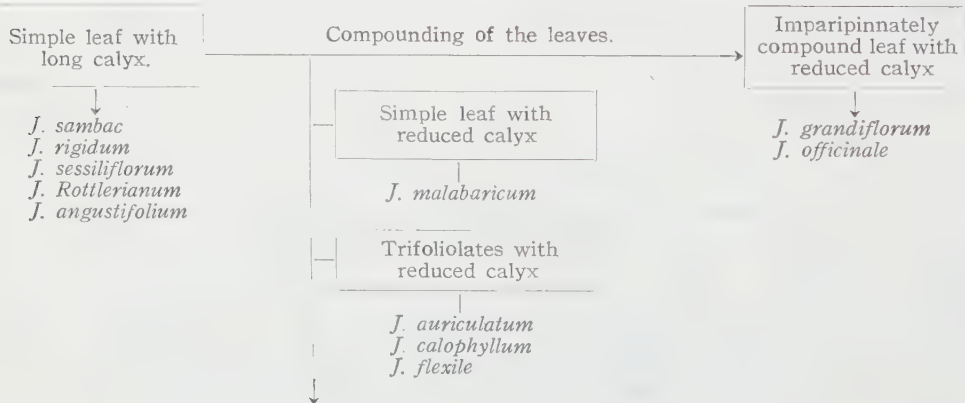
* Part of thesis approved for the degree of Master of Science of the Madras University, 1952.

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and lobes may be telescoped one inside the other. The anthers of the lowermost flower remain normal while those of the upper ones become completely or partially petaloid. Type-IV-fasciation found in stems leads to formation of single large flower with numerous bracts, calyx teeth, anthers and stigma. The flower often assumes a flattened appearance as in a cox-comb. It is interesting that the multiplicity of petals in the flowers has no relationship with the ploidy of the variety concerned. The following gives a schematic representation of the lines of differentiation of corolla in *J. sambac*.

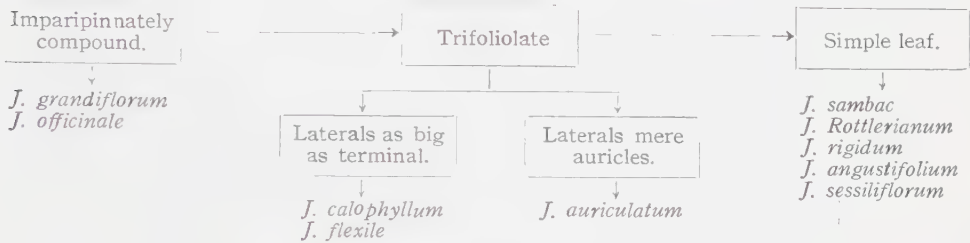


c) Considerations regarding the evolution of plants with simple, trifoliate and compound leaves. Of the different taxonomic criteria employed in the classification of the *Jasminums*, the character of the calyx is an important one (Appendix I). In the evolution of the *Jasminums*, it appears, as though coupled with the compounding of the leaves there has also been differentiation of the calyx.

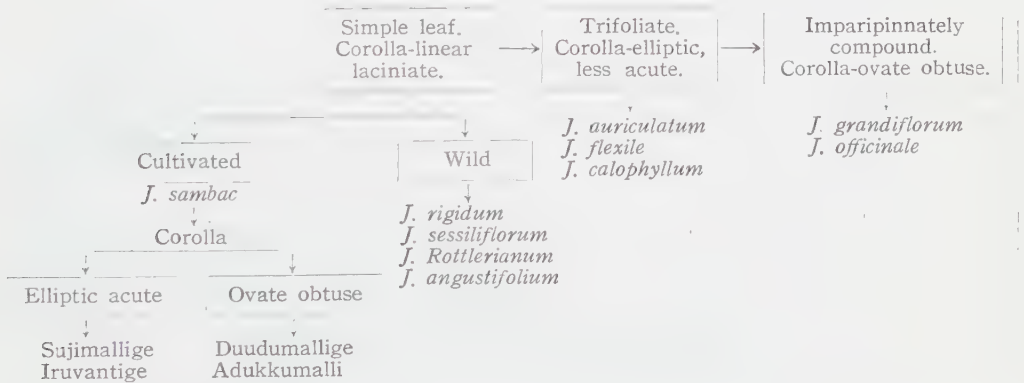


Arber (1950) cites a number of cases in which a compound ternate leaf clearly reveals its equivalence to a simple leaf. From this, it follows that in foliar development the compound leaf is derived from a simple one. In the *Jasminums* somewhere in the direct line of evolution from the simple to the imparipinnately compound leaf, the trifoliolates have been differentiated coupled with reduction in the length of the

calyx through such simple leaved intermediates as exhibit a reduction in the length of the calyx (*J. malabaricum*). Engler and Prantl (l.c) consider the simple leaf as only the transformed terminal leaf-let of the imparipinnately compound leaf, and on this basis species with imparipinnately compound leaves must be considered to be primitive and the simple leaved ones as advanced as shown below:—



Taking the corolla lobes, the evolution may be represented as follows. In the simple leaved (wild) species the corolla lobes are linear and almost laciniate with acute tip. In the trifoliolates they are elliptic and less acute, while in the imparipinnate ones they are more ovate and obtuse. In the cultivated varieties, especially, the selection seems to have been towards the broader ones. In the varieties of *J. sambac*, these three grades of corolla differentiation are observed. Taking the simple leaved (wild) species with linear laciniate corolla lobes as more primitive, the scheme of evolution may be.



The chromosome morphology of the simple leaved species bears more resemblance to those of the trifoliolates rather than to those of the compound leaved ones. The simple leaved species are numerically greater than the trifoliates and hybridization between the two has not been successful.

IV. Summary: The species *sambac* includes two groups of plants, one with elliptic leaves and lanceolate corolla lobes, and another with ovate leaves and corolla lobes.

Differences in morphological characters were observed in plants (growing wild

Table 1. Varieties and

Serial Number.	Name of Species.	Cultivated (-) or Wild (+)	Chief Characteristics	Name of Variety.
1	2	3	4	5
1	<i>J. sambac</i> Ait.	—	Group A. Leaf: simple-opposite-elliptic-dark or yellow green-Flower buds conical. Lobes-elliptic to oblong-acute-Single whorled-rarely two. Corolla tube shorter than lobes-rarely two.	var i Sujimallige (Kannada)
				var ii Iruvantige (Kannada)
			Group B. Leaf. simple-opposite-ovate or ovate elliptic-dark-green. Flower buds globose-lobes oblong to orbicular.	var iii Dundumallige (Kannada)
				var iv Elusuthu mallige (Kannada)

forms of *J. sambac*.

Characters of the variety.	Name of Form	Characters distinguishing form variety.	2n	Floral abnormality type	Fig. No.	Remarks.
6	7	8	9	10	11	12
Leaf-yellow green. Lobes 1.5-2.5 cms long. 0.4-0.6 cm broad	—	—	26	Normal	1	Sets fruits and Seedlings raised.
	form i	Internodes, petioles bracts and calyx-purple. Leaf-dark green.	26	"	—	
	form ii	Leaf-dark-green-lobes ovate.	26	"	—	
	form iii (Kasturimalli)	Corolla lobes very long. 4-4.5 cms long/ 0.4-0.6 cm broad.	26	"	15a	
	form iv (Manmatha-banam)	Leaf-dark green-lobes-intermediate in length between var i & form iii	26	"	—	
	form v	Two whorls of corolla. inner unfolding late	26	Type I	—	
Leaf-dark-green-Telescoping of flowers lobes 1.8-2 cms long & 0.8 cm broad.			26	Type III	2	Virupakshi (Tamil)
One to two whorls of corolla			39	Type I	3 15b	Gundumalli (Tamil)
	form vi (Moonaduk-kumalli)	Corolla lobes many whorled-anthers becoming petaloid	26	Type II		
Leaves in whorls-flowers rose like			26	Type IV	4	Adukkumalli. (Tamil)

Table 2.

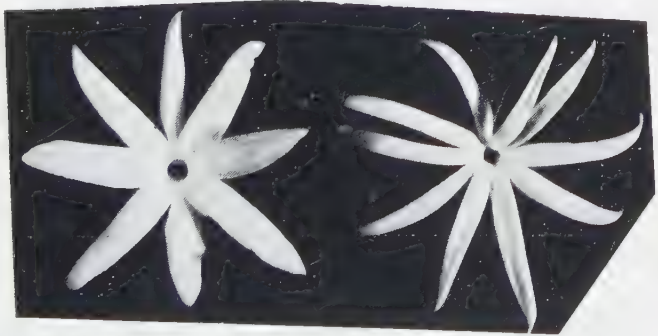
Sl. No.	Name of species.	Cultivated (-) wild (+)	Characters.		2n number.	Fig. No.	Remarks.
			wild (+)	Cultivated (-)			
1	2	3	4	5	6	7	8
1	<i>J. Rottlerianum</i> Wall.	+			26		1-4 Simple leaved species. Seedlings raised in all these.
2	<i>J. malabaricum</i> Wt.	+			26	5	5-Trifoliolate and 6 & 7 imparipinnately compound leaved species.
3	<i>J. sessiliflorum</i> , Vahl.	+			26	6 & 15c (left)	Seedlings raised from 5. Commonly known as jaji and sets fruits (6).
4	<i>J. angustifolium</i> , Vahl.	-	Descriptions not given as these agreed with those given in the floras.		52	8 & 15c (right)	
5	<i>J. calophyllum</i> Wall.	+			26	13	
6	<i>J. grandiflorum</i> Linn.	-			26	14	
7	<i>J. officinale</i> Linn.	-			26		
8	<i>J. rigidum</i> Zenk.	+ & -	Leaves, elliptic to elliptic ovate-lanceolate-6 cm / 2.5 cms. corolla lobes 1.5 cm / 0.5 cm.	Leaf. Cordate - ovate - acute to acuminate - 7.5 - 8 cms long 6 cms broad. Corolla lobes. 1.8-2 cms / 0.5 cm.	+ & - = 26	- = 7	
9	<i>J. auriculatum</i> Vahl.	+ & -	Leaf. Terminal - ovate 3 cms / 2 cm, laterals are auricles - 1 cm / 0.5 cm corolla lobes 0.9 cm / 0.2 cm.	Terminal ovate 6-7 cms / 3.5 cms, laterals 2 cms / 1 cm. Lobes 1.2 cm / 0.4 cm.	+ & - = 26	+ = 9 - = 10	(-) is commonly known as Mullai-dose not set fruits. (+) seedlings raised.
10	<i>J. flexile</i> Vahl.	+ & -	Leaf. Elliptic-acute to acuminate-Terminal 10 cms / 6 cms. laterals 9 cms / 5 cms. corolla lobes-1.5 cm / 0.7 cm.	Elliptic-acute to acuminate-Terminal 5.5 cms / 2.5 cms, laterals 4 cms / 2 cms lobes 1.8 cm / 0.6 cm.	+ - 26 - = 52	+ - 11 - = 12	(-) is commonly known as Ramabanamulai. Flowering throughout, sets fruits. Seedlings raised. (+) seedlings raised.



15a



15b



15c

and cultivated) of *J. rigidum*, *J. auriculatum* and *J. flexile*.

The seedlings of the cultivated *J. flexile* showed mendelian segregations for leaflet size and shape. Four types of floral abnormality in *J. sambac* varieties are described as also their probable method of origin and differentiation.

The relationship between the simple, trifoliate, and imparipinnately compound leaved species and their origin are discussed in respect of the leaves, calyx and corolla lobes.

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Appendix I

Classification of the Jasminums as given by different Systematists.

1. Walpers C.G. (1852) Annals Botanices Systematicae, Vol. 3.
 Section I-Unifoliata
 i) Calyx lobes subulate and elongate
 ii) Calyx subtruncate
 Section II-Trifoliata
 i) Calyx shortly dentate
 ii) Calyx laciniate
2. Engler and Prantl (1897).
 Section I-Unifoliata
 Section II-Trifoliata
 Section III-Alternifolia (Leaves cut up into three or more leaflets.)
 Section IV-Pinnatifolia (Leaves imparipinnately compound)
3. Hooker J.D. (1882) Flora of British India II 590 Reeve & Co. London.
 Group I. Leaves simple, calyx pubescent.
 (This section proceeds from the species with long to those with short calyx teeth)
 Group II. Leaves trifoliate.
 Group III. Leaves imparipinnately compound.
4. Gamble (1936)
 Group I. Leaves simple.
 i) Calyx pubescent or glabrous
 ii) Calyx subulate or short
 Group II. Leaves compound.
 i) Leaves trifoliate
 ii) Leaves imparipinnately compound

Transmission Rate of Photoperiodic Stimulus in *Pharbitis Nil*.

by Shun-ichiro IMAMURA* and Atsushi TAKIMOTO*

今村駿一郎・滝本 敦： アサガオにおける日長刺激の移動速度

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Soon after the discovery of photoperiodism, Garner & Allard, working with *Cosmos sulphureus*, a typical short-day plant, demonstrated that a continuously darkened portion of a plant exhibited some response when an adjacent portion of the same plant was exposed to short day, indicating some transmission of the short-day stimulus to the portion that was kept in darkness⁴). Knott working with spinach, a long-day plant, found that long-day treatment of the leaves induce flowering, even if the bud was exposed to short day. He concluded that some substance or stimulus produced in the leaves must be transported to the growing point causing initiation of floral primordia⁹). Since then many investigations have been concerned with the transmission of flower inducing stimulus and many important results have been obtained^{1, 5, 6, 10, 12, 13}). But as to the transmission rate of the stimulus only a few reports are known. So far as the present authors are aware, the papers by Cajlachjan are the only reports, in which the numerical value of the transmission rate is mentioned^{2, 3}). The purpose of the present investigation was to fill this gap in our knowledge of the physiology of flowering.

Materials and Methods

The strain "Violet" of *Pharbitis Nil* was used in the present research. The photoperiodic behavior of this strain was briefly summarized in previous papers⁹). Upon removal of the main axis the axillary bud of the uppermost leaf begins to develop. If such a plant is transferred to short day condition, floral initiation occurs on the developing axillary shoot. In most cases the growing point of the shoot gives rise to flower primordia from a certain node upward in acropetal succession, leaving several buds at the base in vegetative state. The position of the node which bears the first flower primordium varies, provided other conditions remain the same, according to the time elapsed from the topping of the main axis to the beginning of dark treatment. The later the treatment is started the higher is situated the node, on which the first flower appears. This is due to the fact that

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more basal buds of the developing shoot are determined irreversibly as vegetative when the period between the removal of the main axis and dark treatment is increased and do not respond any more to photoperiodic stimulus. An example is represented in Table 1. On July 7th, 1949 the seeds were sown in a seed bed under

Table 1. Position of the first flower primordium on the developing receptor shoot in relation to the delay of the start of a single dark period of 16 hours' duration after removal of the main axis.

Time in days elapsed between topping and dark treatment.	Distribution of the first flowers on the receptor shoots.								Position of the 1st flower primordium, as indicated by the average number of nodes.
	Number of the nodes.								
	I	II	III	IV	V	VI	VII	VIII	
0	0	4	16	0	0	0	0	0	2.80±0.092
1	0	0	10	10	0	0	0	0	3.50±0.114
2	0	0	0	0	15	4	1	0	5.30±0.128
4	0	0	0	0	2	3	10	3	6.78±0.207

continuous illumination. After germination 100 seedlings were selected for uniformity and divided into five groups. 20 plants of each lot were planted July 12th in a box measuring 32 cm×24 cm×12 cm in four rows of 5 plants each, set at equal spaces between them. On July 29th the plants had two fully expanded leaves. The main axis was cut off above the second node, and all leaves and buds, except the second leaf and its axillary bud, were removed. Then, one lot of 20 plants was immediately subjected to a single dark treatment of 16 hours' duration. 3 other lots were treated in the same way 24, 48 and 96 hours later, respectively. The remaining lot served as control and received no dark treatment. The experiment was carried out in the greenhouse, where the temperature varied considerably. The treated plants were returned to continuous illumination by natural day light supplemented with incandescent electric light of 60 watt hanging 50 cm. above the leaf surface. Luminosity at night was ca. 100 foot candles. After 19 days the plants were collected and examined for flower initiation. All the control plants had no flower primordia and remained strictly vegetative. 4 plants of the lot, which received dark treatment immediately after topping, initiated the first flower primordium on the second node and the remaining 16 plants on the third node. Thus on the average the position-number of the node on which the first flower primordium appeared was 2.80. In the second lot half of the plants initiated flower primordia on the third node and the other half on the fourth. On the average the position-number of the node was 3.50. The lots, which received dark treatment after 48 and 96 hours from the topping had the first flower on the 5.30th and 6.78th node respectively. These positions of the first flower show to what extent the receptors had advanced in their development before they received the stimulus transmitted from the donor

leaf. Therefore the position of the first flower primordium can be used as a measure of time, in which the stimulus reaches the receptor bud with enough intensity to cause floral initiation.

For the estimation of the transmission rate two branched plants were used. They were obtained by clipping the plumule in early seedling stage and forcing the two buds in the axis of the cotyledons to develop, which otherwise remain dormant. After the plants attained the size desired, the axes of both cotyledonary shoots were removed. The uppermost fully expanded leaf of one branch was used as donor leaf and all other leaves were removed. All the buds were also removed except that at the uppermost node of another shoot, which served as receptor bud. The plants were immediately transferred to the dark room from 4 p.m. to 8 a.m. of the next day and this was repeated for 5 days. In time-measuring lots the uppermost fully expanded leaf and its axillary bud were used as donor and receptor, all other leaves and buds having been removed. Dark treatment was given in the same way as in the experimental plants, immediately, one day, two days etc. after the removal of the main axis. After the treatment they were grown under continuous illumination for 14 days or more, thereafter the position of the first flower of the experimental plants was compared with that of the time-measuring plants.

Experimental results

Experiment 1. On June 29th, 1951 seeds were sown in the garden under continuous illumination supplemented with electric light at night. On July 3rd 180 seedlings were planted in nine boxes of 20 plants each and by removal of the main axis on July 8th cotyledonary shoots were forced to develop. On July 30th 50 plants with two well developed cotyledonary shoots were selected for experimenting. As the two cotyledonary shoots developed seldom with equal vigor and were often different in length, leaf number and so forth, 50 plants were divided in two groups. In one group (a), two branches were relatively equal in their development, but in another group (b), they were so different that it seemed reasonable to treat them as a separate group. From the remaining plants 64 were used in 4 time-measuring lots of 16 plants each, and 33 plants as light control. The remaining plants which developed only one cotyledonary shoot were discarded. As the plants of different lots grew in one box, dark treatment of 16 hours' duration for 5 days was given by enclosing the donor leaf blade with a bag of light-proof paper. Time required for the enclosing procedure was at most ca. 30 minutes.

Group (a): This group consisted of 25 plants with relatively equally developed cotyledonary shoots. The second leaf of one branch served as the donor leaf and the second bud of another branch as the receptor bud. On the average the length of the stem of the donor branch was 58.8 cm., that of the petiole 80.0 cm. and of the stem of the receptor branch 74.3 cm. 21 days after the start of treatment the shoots

developed on the second node of the receptor branch were examined. The result is shown in Table 2 a-c. At the end of the experiment 22 plants could be evaluated, three plants were discarded because of the damage suffered by the petioles in the course of the experiment*. The first flower appeared in one plant on the 2nd, in 4 plants on the 3rd, in 6 plants on the 4th and in 11 plants on the 5th node. The average position-number of the node, on which the first flower appeared was 4.23.

In the time-measuring lots the plants treated immediately after the removal of the main axis initiated the first flower primordium on the 2.53th node on the average. In the plants treated 24, 48 and 72 hours later, the first flower primordium

Table 2a Position of the first flower primordium on the receptor branch, induced by a single donor leaf on another branch in two-branched plants. Experiment 1.

Group	Number of plants observed	Length of the path in mm				Average position indicating number of the 1st flower primordium
		Petiole of the donor leaf	Stem of the donor branch	Stem of the receptor branch	Total	
(a)	22 ¹⁾	80.0 \pm 2.86	58.8 \pm 3.38	74.3 \pm 3.52	213.1 \pm 4.74	4.23 \pm 0.197
(b)	23 ²⁾	99.9 \pm 2.26	22.0 \pm 1.24	84.9 \pm 2.40	205.9 \pm 3.90	4.78 \pm 0.140

Table 2b. Position of the first flower primordium in relation to the time elapsed from topping to the start of short day treatment. Time-measuring lots.

Time elapsed from the topping to the start of dark treatment in hours.	0 ³⁾	24 ³⁾	48 ³⁾	72 ³⁾
Number of plants observed	15	15	14	16
Petiole length in mm.	90.9 \pm 3.10	88.5 \pm 4.01	93.8 \pm 3.26	95.1 \pm 2.71
Position of the first flower	2.53 \pm 0.247	3.73 \pm 0.228	5.14 \pm 0.143	6.25 \pm 0.266

Table 2c. Calculation of transmission rate.

Group	Average path length of experimental plants in mm.	Average path length of time-measuring plants in mm.	Difference in path length in mm.	Retardation of stimulus in hours	Transmission rate in mm. per hour
(a)	213.1	91.2 ⁴⁾	121.9	32.5	3.8
(b)	205.9		114.7	41.9	2.7

1) donor: second leaf, receptor: second bud.

2) donor: first leaf, receptor: second bud.

3) donor and receptor on the second node.

4) The petiole lengths of two time-measuring lots, between which the interpolation was done, were different, their average value was used for calculation:

$$(88.5+93.8)/2=91.2$$

* The petioles were damaged also in some plants of other lots, and such plants were excluded from observation.

appeared on the 3.73th, 5.14th and 6.25th node, respectively. From this results we may conclude that in the experimental lot the flower initiating stimulus reached to its receptor bud later than in the 24 hour lot but earlier than in the 48 hour lot. By interpolation we could calculate when the dark treatment of the time-measuring plants should be started to secure the same position of the first flower as in the experimental lot. It was calculated to be 32.5 hours after the removal of the main axis. The delay of the arrival of the stimulus to the receptor bud is due to the difference in the path length, which the stimulus must traverse in the experimental and in the time-measuring plants.

To calculate the average velocity, it was assumed that the stimulus, regardless of the nature of the organs concerned — stem and petiole —, and of the direction — upward and downward —, is transmitted with equal velocity. The difference of the total path length between the experimental and the time-measuring lots was 121.9 mm. So we obtain as average velocity $121.9/32.5$ i.e. 3.8 mm. per hour.

Group (b): This group consisted of 25 plants with relatively unequally developed cotyledonary shoots. As the receptor bud the second axillary bud on the longer shoot and as the donor leaf the first leaf on the shorter shoot were used. In the time-measuring lots the second leaf of the longer shoot was used as donor and its axillary bud as receptor. The average position of the first flower primordium was 4.78, and the delay of the arrival of the stimulus as compared with the first and second of the time-measuring lots was 41.9 hours. The difference of the total path length between the experimental and the time-measuring plants was 114.7 mm. The velocity of transmission was therefore 2.7 mm. per hour on the average.

Experiment 2. On the 15th of June 1952 the seeds were sown and on the 20th 160 plants were planted in eight boxes, 20 plants in each, and two-branched plants were obtained in the same manner as described above. The experiment was started on the 15th of July.

Group (a): 40 plants with relatively equally developed cotyledonary shoots were selected for one experimental group. The 2nd and 3rd leaves of the shorter branch were used as donors and the 3rd or 4th lateral buds as receptors. Position of the first flower was 4.61 on the average of 38 plants examined, as shown in Table 3a.

3 lots of 20 plants each were used as the time-measure. The 3rd or 4th leaf and their axillary buds were left intact as donor and receptor. 20 plants received no dark treatment and remained strictly vegetative. The position of the first flower in the plants treated 24 hours after topping was 3.38. Those of the time-measuring plants treated 48 and 72 hours later were 4.50 and 5.30, respectively. The delay of the arrival of the stimulus to the receptor bud in the experimental lot as compared to the time measuring lot was 51.3 hours. The difference in the path length was 147.6 mm. and we could calculate the velocity as 2.9 mm. per hour.

Table 3a. Position of the first flower primordium on receptor branch, induced by a single leaf on donor branch. Experiment 2.

Group	Number of plants observed	Leagth of the path in mm				Average position indicating number of the 1st flower primordium
		Petiole of the donor leaf	Stem of the donor branch	Stem of the receptor branch	Total	
(a)	38 ¹⁾	100.9±1.37	37.1±2.59	97.8±3.99	235.8±6.46	4.61±0.163
(b)	23 ²⁾	122.3±4.58	11.8±0.78	98.7±4.09	232.9±7.62	4.79±0.202

Table 3b. Position of the first flower primordium in relation to the time elapsed from topping to start of short day treatment. Time-measuring lots.

Time elapsed from topping to start of dark treatment in hours	24	48	72
Number of plants observed	18 ³⁾	20 ⁴⁾	20 ⁵⁾
Petiole length in mm.	88.8±3.94	90.7±4.23	85.6±4.85
Position of the first flower	3.38±0.211	4.50±0.154	5.30±0.193

Table 3c. Calculation of transmission rate.

Group	Average path length of experimental plants in mm.	Average path length of time-measuring plants in mm.	Difference in path length in mm.	Retardation of stimulus in hours	Transmission rate, in mm per hour
(a)	235.8	88.2	147.6	51.3	2.9
(b)	232.9		144.7	56.7	2.6

- 1) donor: in 26 plants the second, in other plants the third leaf. receptor: in 30 plants the third, in other plants the fourth bud.
- 2) donor: the first leaf, receptor: the third bud.
- 3) donor and receptor: in 9 plants on the third, in other plants on the fourth node.
- 4) donor and receptor: in 8 plants on the third, in other plants on the fourth node.
- 5) donor and receptor: in 10 plants on the third, in other plants on the fourth node.

Group (b): 25 plants with unequally developed branches were used in this group. The first leaf on the shorter branch was used as donor and the 3rd bud on the longer branch as receptor. The position of the first flower primordium was 4.79. The delay amounted to 56.7 hours and the difference in the path length was 144.7 mm., so the velocity of transmission was 2.6 mm. per hour.

General consideration

It may be admitted that the experiments mentioned above have some error sources. All the buds and leaves of the donor and receptor branches of all experimental and time-measuring plants should be strictly comparable concerning their physiological condition in order to obtain a convincing result. This was not strictly

the case in the above experiments, especially when the two cotyledonary branches differed conspicuously in their development. The sensitivity of the donor leaf and the morphogenic reactivity of the receptor bud may have been different in the experimental and the time-measuring lots. The leaves of the experimental plants were often older than those used for time-measure. Concerning this point group (a) of experiment 1 is the most reliable, since the difference was the least.

The calculation was made under the assumption that the stimulus travels in stem and petiole, upward and downward, with equal velocity. But a preliminary experiment showed that the stimulus seems to move acropetally more easily than in the reverse direction. Regardless of the elements of uncertainty contained in the experiments, we may conclude as an approximation that the floral stimulus may be transmitted with an average velocity of 2-4 mm. per hour. This value is large in comparison with that obtained by Cajlachjan in *Perilla*. He has obtained values approximating 2 cm. in 24 hours in stems and 0.5 cm. in 24 hours in roots. In citing his work Lang states: "It is however, uncertain whether the observed rates were optimal, since in the experiments the length of the transport route (down and up a whole stem, split lengthwise) may have been out of proportion to the size of the supplying area (a single leaf)"¹¹⁾. To our great regret, his works were not available to us, so we cannot decide whether the discrepancy is due to the difference in the experimental methods employed or to the difference of the plants used. The latter alternative might not be excluded as various plants are remarkably different in the ease with which the floral stimulus is transmitted.

Summary

Under the assumption that the photoperiodic stimulus travels with uniform velocity not only in petiole and stem, but also in acropetal and basipetal direction, the average rate of transmission was found to be 2.6-3.8 mm. per hour.

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Studies on the Leaf Nodules V

The Formation of Knobs by the Effect of Antibiotics on the *Ardisia crispa* and *A. punctata*

by Tamotsu YAMADA*

山田 保: 抗生物質による葉瘤植物の瘤の形成

Received June 21, 1955

I. Preface

In the preceding report (1954) the author published the results of the effect of heat treatment on the *Ardisia crispa* and the *A. punctata*; in this paper the influence of antibiotics on them especially the knob formation will be described.

Miehe (1929) and the author (1953) reported that when the seeds of the *A. crispa* (Manryo) and the *A. punctata* (Karatachibana) which are the two only species of leaf-nodulated plant growing in Japan are treated with heat (45-55°C), some seedlings from them grow into non-leaf-nodulated plants, and the non-leaf-nodulated plants and some of the others come to have knobs (Höcker) on their various growing points of the plants and after that the growth of them stop.

We have observed that if the seeds or seedlings just after germination are treated by antibiotics, some of them grow into perfect non-leaf-nodulated plants and knobs are made on the growing points as in the case of the heat-treated plants, and all these knobs formed by antibiotic treatment can not be distinguished from those formed by heat-treatment. We are going to report briefly on the aquired results.

With much gratitude I mention here the kindness of Mr. S. Iai, M. Miyama and N. Watanabe in photographing and valuable suggestions.

II. Material and Methods

The seeds of the *A. crispa* and *A. punctata* were collected in December, 1952 and preserved in dry sand, and in May-July, 1953 they were used as materials after peeling. Solution of various concentration i.e. the 1000-5 γ solutions per cc of streptomycin (St), aureomycin (Au), chloromycetin (Ch) and terramycin (Te) were used in the following methods. 1) Seed treatment: Non or barely germinated seeds of the two species were immersed for 3-7 days in the above mentioned antibiotic solution in sterilized dishes, and then they were sowed in flower-pots by the usual method. 2) Growing point treatment: In an early period of germination

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when the cotyledon had not developed, treated the growing points with each antibiotic of the above mentioned, absorbed in absorbent cotton, covering them with beakers and then changed the cotton every seven days, repeating this procedure four times in all, and after 16 months observed the result.

III. Experimental Results

1. Seed treatment: As shown in Table 1, in the case of the *A. crispa*, the number of the plants on which knobs were formed by the effect of antibiotics increases in proportion as the concentration of antibiotic solution is higher and the duration of immersing becomes longer, for instance, the 1000 γ solution is the most effective, and when the 7 day-treatment is compared with the 3 day-treatment, the number of the plant on which knobs were formed in the former case is two times as many as that in the latter. In the case of the *A. punctata*, germination was

Table 1. Formation of the knobbed plants as affected by antibiotics to the non-germinated seeds of the *A. crispa* and *A. punctata* (%).

Treatment Concen- tration Antibiotic		3 days				7 days			
		A	B	C	Average	A	B	C	Average
<i>A. crispa</i>	St	33.3	0	20.0	17.8	100.0	42.9	50.0	64.3
	Au	20.0	14.3	0	11.4			50.0	16.7
	Ch	33.3	42.9	0	25.4	100.0	100.0	75.0	91.7
	Te	100.0	28.6	75.0	67.9	90.0	80.0	25.0	65.0
	Total average	46.67	21.45	23.75	30.63	72.50	55.73	50.00	59.41
	Control				0				0
<i>A. punctata</i>	St		100.0		33.3				0
	Au				0			50.0	16.7
	Ch	0	100.0	0	33.3	100.0			33.3
	Te				0		100.0		33.3
	Total average	0	50.00	0	16.67	25.00	25.00	12.50	20.83
	Control				0				0

A=1000 γ B=500 γ C=250 γ (per cc)

very bad but the above mentioned relations in the *A. crispa* could also be recognized. Through these experiments, it is clear that Ch, Te and St are effective on the formation of knobs, and knob-forming plants are more in the *A. crispa* than in the *A. punctata*, and this phenomenon was also found in the case of heat treatment.

When radicles of the *A. crispa* elongate about 0.5 cm long and treat the seeds in the same way as mentioned above, the result is obtained which is shown in Table 2, but in this case, many of them treated for 7 days, died before the shoots sprung up from the ground, but in the case of the seeds treated for 3 days, knob-



Fig. 1—3.

- 1. Knobbed *A. punctata* (upper) and *A. crispa* (lower) caused by streptomycin.
- 2. Knobbed *A. crispa* caused by chloromycetin.
- 3. Non-leaf-nodulated *A. crispa* caused by streptomycin.

Table 2. Formation of the knobbed plants as affected by antibiotics to the just germinated seeds of *A. crispa* (%).

Antibiotic \ Treatment Concentration		3 days				7 days			
		A	B	C	Average	A	B	C	Average
<i>A. crispa</i>	St		100.0	100.0	66.7			0	0
	Au	0	100.0	33.3	44.4	0		20.0	6.7
	Ch	100.0	0	0	33.3			0	0
	Te			100.0	33.3		100.0	100.0	66.7
	Total average	25.00	50.00	58.33	44.44	0	25.00	30.00	18.33
	Control				0				0

forming plants were produced in the inverse ratio to the degree of the concentration of the antibiotic solution. The reason for this phenomenon seems that the death of the seedlings was caused by the too strong action of higher concentration of the antibiotic solution and long duration of treating time.

2. Growing point treatment: In the *A. crispa*, the number of knob-forming plants by the treatment grows larger in proportion as the concentration of antibiotic solution becomes higher as shown in Table 3. In this case, the 1000 γ solution of each antibiotic is the most effective for the formation of knobs but the 5 γ solution

Table 3. Formation of the knobbed plants as affected by antibiotics by the growing point treatment to the *A. crispa* and *A. punctata* (%).

Antibiotic \ Concentration		A	B	C	D	E	F	G	H	Average
<i>A. crispa</i>	St	100.0	0	85.7	100.0	87.5	50.0	37.5	0	57.59
	Au	66.7	62.5	85.7	10.0	20.0	6.7	0		31.45
	Ch	100.0	50.0	0	25.0	33.3	28.6	0	0	29.61
	Te	80.0	90.0	83.3		0	0	16.0		33.36
	Total Average	86.68	50.63	63.68	33.75	35.20	21.33	13.18	0	38.08
	Control									0
<i>A. punctata</i>	St	100.0	50.0			100.0	0	100.0	0	43.75
	Au		85.8	100.0	0	0		0	0	23.23
	Ch	50.0	33.3	60.0		33.3	0	0	66.7	30.41
	Te	25.0	0	40.0	0	71.8	0	0	0	17.10
	Total average	43.75	42.28	50.00	0	51.28	0	25.00	16.18	28.62
	Control									0

D=125 γ E=60 γ F=30 γ G=15 γ H=5 γ (per cc)

of any antibiotic yields no knob. Streptomycin was the most effective of all and the plants having knobs amount to 57.59% in average.

In the other hand, in the case of the *A. punctata* the above mentioned relations were not distinctly recognized and this seems to have resulted from the death of many plants after the treatment, but it is presumable, however, that streptomycin is the most effective as in the case of the *A. crispa*. Through these experiments a few non leaf-nodulated plants grow in the case of the *A. crispa*. On the *A. punctata* some non-leaf-nodulated leaves were got for the first time by this method and knobbed plants amounting to 66.7% were got exceptionally by the treatment of the 5 γ solution of Chloromycetin.

3. Anatomical structure of knobs formed by the treatment of antibiotics: As already mentioned, when the seeds or the growing points of the *A. crispa* and the *A. punctata* are treated by some antibiotic solution, knobs are formed on each growing point in the next year. To these knobs, the author has given the following names for the sake of convenience apical, axillary, and cotyledon knob by the part of its formed.

The knobs of the *A. crispa* formed by the treatment of antibiotic are round, ellipse or irregular in shape, just like those formed by the treatment of heat, and are larger than those of the *A. punctata*, and the color is dark green or greenish brown. In the case of the *A. punctata* the knobs are round and not ellipse, and smaller than those of the *A. crispa* and the color is deep green or green. This color comes from chloroplast contained in the cell of outer cortex, and from the color of epidermis itself.

By fixing and staining the knobs, we can see that the outer part of the knob is covered with a single layer of epidermis and the main part of them is filled with many parenchymatous cells of cortex and a central cylinder as Fig. 4-8. It can safely be said that knob-formation begins with the projection of a central cylinder upwards just like the development of lateral root, and then epidermis and cortex of the stem attach themselves around it and becomes larger mainly by the enlargement of the cortex cells, and thus a knob completes itself. Sometimes, knobs are made by the inhibition of the development of primordial leaves of various growing

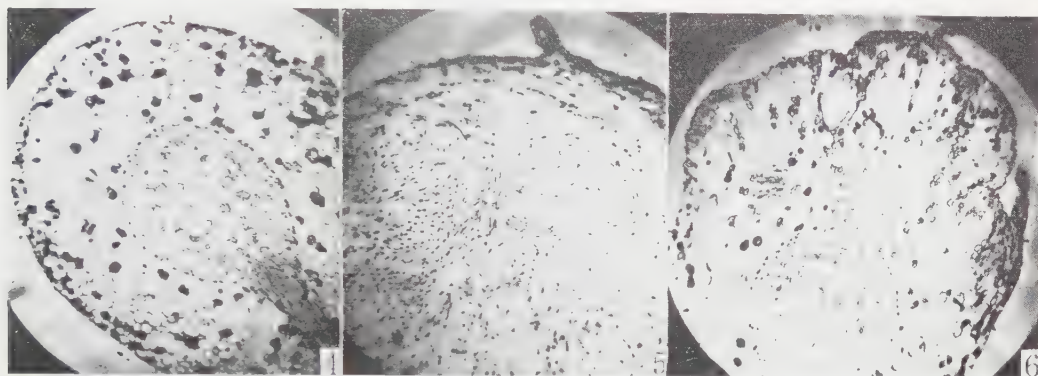


Fig. 4-6.

4. Longitudinal section of an apical knob of *A. crispa* caused by streptomycin. ca $\times 70$
5. Cross section of an apical knob of *A. crispa* caused by chloromycetin. ca $\times 70$
6. Longitudinal section of an axillary knob of *A. crispa* caused by chloromycetin. ca $\times 70$

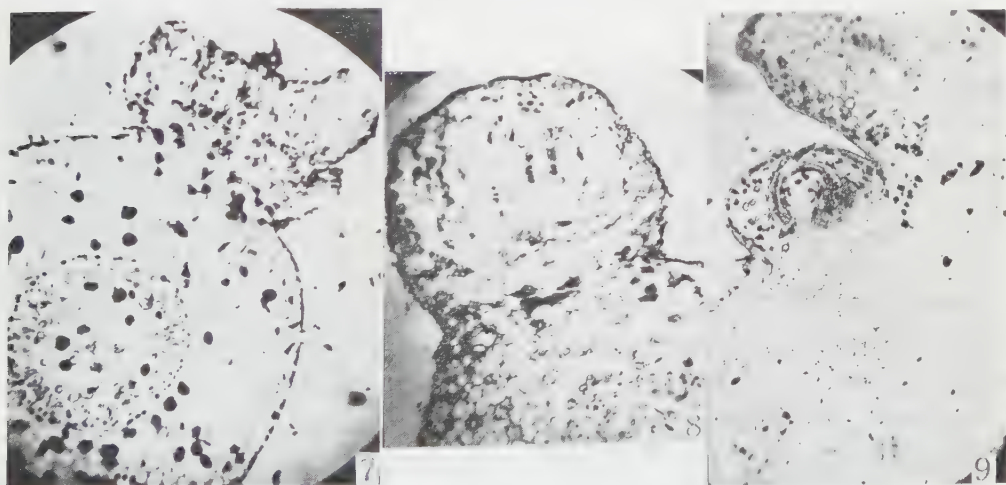


Fig. 7-9.

7. Longitudinal section of an axillary knob of *A. punctata* caused by chloromycetin (upper part). ca $\times 70$
8. Longitudinal section of an axillary knob of *A. crispa* caused by streptomycin. ca $\times 70$
9. Formation of an apical knob of *A. punctata* caused by the thickening growth of the primordial leaves in the treatment of streptomycin, afterwards the growing point and the leaves are sealed by the surrounding tissue and completes a knob. ca $\times 70$

points, in this case the leaves are thickened and cover the growing point, then the just under tissue of the growing point starts the thickening growth and lastly seals the entire growing point and primordial leaves.

No meristem could be recognized in knobs but the cells of the growing point of the stem still exists sealed in knobs, and they seem in some extent to be in the state of cell division. Almost all the outer cells of the knobs are living but the inner ones are dead and cell contents of them are generally scanty.

From the fact that some undifferentiated primordial leaves are attached to the side of the apical part of the knobs or wrap it as Fig. 9, it is presumable that at first the antibiotic prevents the growth and development of the primordial leaves and on the other hand, a central cylinder elongates upwards and then in this process the formation of knobs is promoted. But the fact that the leaf-nodule-bacteria not exist in any parts of them is ascertained microscopically. From these results, it can be decided that the knobs formed by antibiotic treatment coincide with those formed by heat treatment in every respect.

IV. Consideration and Conclusion

As mentioned above, knobs are formed in the *A. crisper* and *A. punctata* when the seeds or growing points of the seedlings are treated by antibiotic solution of various concentration, and the formation of knobs has direct relation to the range of the concentration of the solution and the duration of immersing in it and the effect of the 5 γ solution of each antibiotic, excepting that of Ch, on the *A. punctata* was not utterly acknowledged. On the other hand, as mentioned in the foregoing report, in the case of the heat treatment of the seeds, knobs were formed in proportion to the treatment temperature and the duration of treatment, but by the treatment at 45°C for 10 minutes they were not formed in the both species. From these facts, can say that the formation of the knobs by the both treatments are quite similar. In either treatment and in either species, the knobs are formed from the same origin and the internal structure is also similar, this being recognized clearly by the anatomical investigation of them. And from the fact that neither meristem nor leaf-nodule-bacteria exist in them, it can safely be said that the formation of the knobs is due to the same physiological cause.

The knobs are formed by the treatment of antibiotic or that of heat only in the case of such leaf-nodulated plants as the *A. crisper* and the *A. punctata* which have leaf-nodules in normal condition. But in the case of non-leaf-nodular plant such as the *A. japonica* which is very nearly related to these two species, the knobs are not formed by these treatments. Namely the formation of knobs is the phenomenon observed only in the leaf-nodulated plants. By this result, it can be presumable that the formation of the knobs is related to the existence of leaf-nodule-bacteria, whether they are living or not. Then it is probable that leaf-nodule-

bacteria which had clustered on the growing points die out by the effect of heat or antibiotic treatment and at the time of treatment knob-forming substance may be secreted by the bacteria, which substance stimulates the cell division of the young cells of the growing parts, thus the knobs being formed. It is also suggestible that stimulation substance acts on the formation of leaf-nodules, cooperating with physical stimulation given by bacteria to young leaf tissue. (The knob forming substance will be dealt with in another report afterwards).

V. Summary

1. If the seeds and growing points of the seedlings of the *A. crispa* and the *A. punctata* are treated with each solution of high concentration of streptomycin, aureomycin, chloromycetin and terramycin, knobs are formed on various growing points of the plants and then the growth of the plant stop as in the case of heat treatment, but in the *A. japonica* which is closely affined to them and non-leaf-nodular plant, knobs are never formed either by antibiotics or by heat treatment.

2. Knobs are formed in proportion to the degree of concentration of the solution and the duration of treating time, and in the same method of treatment knob-forming ratio of the *A. crispa* are generally higher than that of the *A. punctata* and the size of the knobs of the former plant is larger.

3. The knobs are formed of epidermis, cortex and central cylinder, and no meristem and leaf-nodule-bacteria are recognized in any part of them by microscope, and the structures of them are almost as same as those formed by the treatment of heat.

4. It is probable that the formation of the knobs is due to a substance, secreted by the leaf-nodule-bacteria, which cluster on the growing point and die by the treatment of antibiotic or of heat, and that this knob-forming substance may also acts on the formation of leaf nodules to some extent.

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Physiological Studies on Growth and Morphogenesis of the Isolated Plant Cell Cultured in Vitro. I

General Feature on the Growth and Morphogenesis of the Internodial Cell of Characeae*

by Tadashi SANDAN**

山段 忠: 遊離植物細胞の生長・成形に関する生理学的研究. I.
車軸藻類節間細胞の生長・成形についての概説.

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The internodial cell of *Chara* or *Nitella* has been used in many experiments as a suitable material for the study of protoplasmic streaming, bioelectric phenomena, osmosis, etc. With respect to the growth and morphogenesis of the internodial cell, however, our knowledge is still meager. Osterhout (1952) mentioned the existence of a longitudinal polarity in the cell, since when it is freed from neighboring long cells and kept at 15°C new shoots appear at one end and roots grow out at the other end. These new growths arise from small cells located at the terminals of the cell.

The present work deals with the behavior of an isolated internodial cell with special reference to growth and morphogenesis. The internodial cell, which is freed from the mother plant, continues its growth and occasionally forms new shoots or rhizoids when it is kept in the native water in which the plant grew, but it can not survive long. When, however, the cell is cultured in the medium which consists of agar gel and the culture solution, it continues its growth and morphogenesis leading to a complete form having 'leaf' cells, 'stem' cells and 'root' cells. It was demonstrated in the present experiments that there exists a morphogenetic polarity in one and the same cell. For the basic culture medium tap water or dilute Knop's solution was used. Besides, dilute IAA-K solution was also applied in the present experiment.

Materials and Methods

The internodial cells of both *Chara coronata* and *Nitella flexilis* were used as material. The length and the width of the material were respectively about 2.5 cm and 500 μ in *Chara*, and 3.0 cm. and 400 μ in *Nitella*. The plants were cultured in a large glass vat in the laboratory. The vat was at first filled with the pond water,

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in which the plant grew, but it was gradually replaced with tap water (pH 6.8). The internodal cell freed from the neighboring cells was placed in distilled water for 24 hours in order to clean its surface and to recover its normal state from possible effects of cutting the adjacent cells, before it was transferred to the test tube or the Petri dish for artificial culture. The test tube is about 2 cm in diameter, 20 cm in length, and the Petri dish is 2 cm in depth and 5 cm in diameter.

The cells were cultured both in vertical and horizontal positions. In the former case, which we hereafter refer to as vertical culture for simplicity's sake, the material was set in a test tube, while in the latter case, horizontal culture, it was set in a Petri dish. There are further two positions in vertical culture according to whether the cell is set in the normal or inverted position.

The test tube and the Petri dish were both filled by half with 0.8% agar. The rest of the tube or dish was filled with the culture solution, i. e. tap water, dilute Knop's solution (1/20 conc. of the original solution) or IAA-K solution (10 mg/500 cc of distilled water). In the vertical culture the material was embedded into agar in such a way that only one end of the cell was in agar, while in the horizontal culture the cell was completely submerged in it. Embedding of the material was carried out soon before the agar gelatinized. These experiments were carried at the room temperature under the diffused light of about 30 lux.

As a directly visible indication of the physiological conditions of the cell, the author paid an attention to the rate of protoplasmic streaming. The rate of flow being very sensitive to the change in temperature, the tube containing the cell was brought into a small water bath kept at 25°C on the stage of microscope when observations were made. The comparative values of the rate of flow were obtained from the period necessary for the particles in cytoplasm to travel a definite distance.

Results

1. The growth and morphogenesis of the cell in vitro

A. Vertical culture

a) Normal position

In the case in which the apical-basal relation is same as that in the natural state, the growth and organ formation of material were most satisfactory. The cell continued to elongate by 8% of the original length after 30 days' culture. In about 15 days after the cell was cultured in vitro, shoots were formed at the apical end of the cell and rhizoids arose from the basal end. These shoots or rhizoids increased their size and number with the result that the material took the complete form consisting of 'leaf' cells, 'stem' cells and 'root' cells. Fig. 1 shows the cell cultured in this manner for 40 days. Rhizoids reached several cm in length but they are not only colorless but also so fine that we could not recognize their clear figures without special caution. The rhizoids in Fig. 1 were stained with chlorzinc

iodine. Though rhizoids were also formed at the apical end of the cell later, they were less and still finer than those developed at the basal end. But when the internodal cell became old and decayed after a long culture, rhizoids at the apical end rapidly increased their size and number and supported new buds developing from the apical end. Thus a new complete plant was formed.

For the formations of shoots and rhizoids IAA-K solution or Knop's solution was more favorable than tap water. The next table shows the processes of elongation of shoot or rhizoid when these culture solutions were applied. According to this table, it is revealed that IAA-K solution and Knop's solution promoted growths of shoot and rhizoid by 40% and 20% respectively as compared to the case of plain tap water.

Table 1. Length of shoots formed at the apical end and of roots formed at the basal end of the internodal cell cultured in vertical, normal position. (cm)

	Culture solution	Days in culture				
		15	30	45	60	90
Shoot	Tap water	0.3	1.2	3.4	3.7	3.8
	IAA-K sol.	0.6	1.9	4.8	5.4	5.9
	Knop's sol.	0.2	1.4	3.9	4.6	4.9
Rhizoid	Tap water	1.1	1.7	2.4	2.6	2.9
	IAA-K sol.	1.6	2.3	3.1	3.6	4.3
	Knop's sol.	1.4	2.0	2.9	3.1	3.6

The values shown in the table are the averages of five experiments in each case.

The materials cultured in this manner continued their growth and differentiation eventually forming antheridia and oogonia in about 150 days.

The above experiments clearly demonstrate the existence of morphogenetic polarity. New shoots always arose from the apical end of the cell, and rhizoids were formed primarily at the basal end. Later rhizoids were also formed at the apical end. These rhizoids, which were formed at the apical end, supported the newly grown-up body of considerable size as the substitutants of the rhizoids formed from the basal end.

b). Inverted position

In the case, in which the cell was kept upside down, a new shoot formed in 15 days at the apical end which was actually set downward. Rhizoids arose in the same duration of time at the basal end which was actually set upward. Rhizoids were formed also at the apical end later. These facts lead us to assume that the morphogenetic polarity is maintained even though the material is set in the inverted position just as well as in the normal position.

When the lower half of the test tube containing material in the inverted position was covered with a black paper in order to keep dark the lower end of the cell,

which is actually the apical end, new shoots were formed from the upper end, which is actually the basal end, after about 15 days (Fig. 3) and at the same time rhizoids were formed at the apical end set downward. Thus the morphogenetic polarity of the cell could be artificially reversed by changing light condition.

B. Horizontal culture

In this case the cell was cultured in horizontal position as shown in Fig. 10 in which A is the region of culture solution and B is the agar region in the Petri dish. The cell formed a new shoot from the apical end of cell and a considerable number of rhizoids from the basal end after about 15 days' culture. They continued to grow for about 50 days. The morphogenetic polarity thus seems to be maintained in this case.

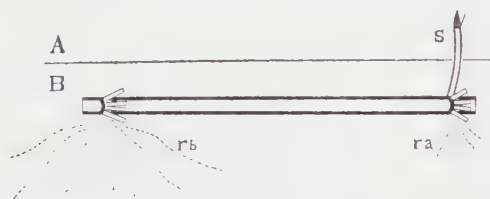


Fig. 10



Fig. 11

As the next step, the operation of strangulation was applied with the purpose of obtaining a single cell in absolute absence of small nodial cells which otherwise may not be removed completely. As in Fig. 11 the cell was tied off at two regions with strips of silk thread. The central fragment of an internodal cell which was strangulated at both its ends was cultured in the horizontal position. In this experiment, we observed that the strangulated cell fragment showed a little increase in length and, furthermore, that a new shoot was formed from the strangulated region of the apical side and rhizoids arose from that of basal side in about 20 days. This new shoot and these rhizoids could survive for more than 50 days. Although a shoot was formed also at the basal side and rhizoids came out from the apical side of the cell fragment later, they were weak and survived for only 10 days. These facts show that the morphogenetic polarity is still maintained to a certain extent in the cell fragment artificially formed by the strangulation.

2. Observation of protoplasmic rotation

As the cell of *Nitella* or *Chara* shows the typical protoplasmic rotation, the measurement of its velocity was carried out in the cell cultured in the vertical, normal position. The measurement was conducted in the manner mentioned above. One example of the results is shown in Fig. 12 in which the rate of flow is plotted against time. Ordinates represent the rate of flow in microns per second, and abscissas time of culture in days.

The figure shows that the velocity of protoplasmic rotation was small for two or three days after cell was transferred to the culture medium. This decrease in

velocity is due probably to the possible mechanical shock accompanying the treatment. It is noteworthy that the rate of flow of protoplasm is obviously accelerated for several days prior to the shooting or to the rhizoid formation. After shooting, the rate came back to the original, constant value again. This promotion in protoplasmic flow seems to show the increased activity of the cell function.

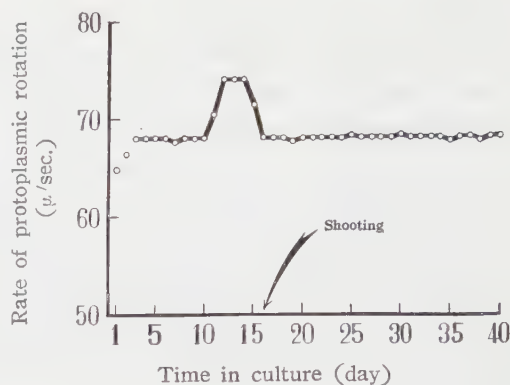


Fig. 3

Discussion

When the internodal cell of *Nitella* or *Chara* was kept in various liquids in vitro such as the pond water in which the plant grew, we observed an only insignificant growth. On the other hand, when it was cultured in the vertical, normal position by the foregoing method using agar, the cell showed a considerable growth and morphogenesis. This fact shows that agar may give some favorable conditions, physically or chemically, to the cell for the formation of rhizoid.

The results of the present work clearly show the existence of the polarity concerning shoot and rhizoid formations. Osterhout (1952) pointed out that the longitudinal polarity of the *Nitella* cell is not altered. The results of the present work, however, demonstrate that this polarity can be reversed by light condition.

It is likely that some substances inducing the formation of shoot or rhizoid may exist in the cell with the apical-basal gradient of concentration. Hämmerling (1953) mentioned that the nucleus of *Acetabularia* controls the pattern of organization, especially of caps, by the production of morphogenetic substance. A similar situation as the one he describes may exist in the cell of Characeae in respect to its morphogenesis.

We are in a position to assume that the substances having an effect upon shooting may move along the cell under certain light conditions, or that these substances may be activated or inactivated through the photochemical reaction. The foregoing experiment showed that only weak light is sufficient for inducing the shoot formation. This is well to be understood in view of the fact that the material usually grow at rather deep place in lakes.

On the contrary, rhizoids were not only formed at the dark region but also at the bright region. Thus we can assume that light is not indispensable to the cell for rhizoid formation. The new rhizoids were also formed later at one end of the cell after the rhizoids were formed at the other end. This fact seems to show that the substances promoting rhizoid formation exist comparatively evenly in the cell. Hence the cell has a possibility of forming rhizoid at either end.

Jacobs (1951) mentioned that the formation of rhizoid in *Bryopsis* was promoted with auxin. In the present work, dilute IAA-K solution promoted formations of both shoot and rhizoid in the internodal cell of Characeae cultured in vitro. The substances which promote shooting or rhizoid formation may have a close relation to IAA.

Protoplasmic rotation in the cell was obviously accelerated at the definite period before shooting. This increased rate of the protoplasmic flow may be due to increased metabolism. It is, however, noteworthy that a decrease in viscosity of protoplasm was ascertained, an increase in the rate of flow being observed in the cell.

Linsbauer (1929) observed that the internodal cell of Characeae, which was tied off into two cell fragments, survived for 60 days. In his experiment the strangulated internodal cell was cultured in a Petri dish filled with well water and kept at the room temperature under the diffused light. But in his case the cell showed no morphogenetic change in spite of containing such cell components as nuclei, chloroplasts, plenty of reserve materials, etc. He assumed that the strangulated internodal cell was already developed fully and it had no more morphogenetic ability. In the present result, the cell, which was artificially tied off at both its ends, not only increased its size but also formed new shoots and rhizoids. Linsbauer probably failed to observe the morphogenetic development of the cell due to his inadequate culture method.

The author wishes to express his best thanks to Prof. N. Kamiya of Osaka University for his kind direction and helpful criticism throughout this work and also to Prof. T. Nakamura for his valuable advice.

Summary

1. The internodal cell of *Chara* or *Nitella* which was freed from adjacent cells was cultured in vitro for a long time in the following media with 0.8% agar: tap water, dilute Knop's solution (1/20 of the original conc.), and dilute IAA-K solution (10 mg/500 cc of distilled water). The cell thus cultured showed not merely its growth but morphogenesis.

2. The morphogenetic polarity concerning the formations of both shoot and rhizoid was ascertained. This morphogenetic polarity was reversed by changing the light condition.

3. The cell fragment, which was artificially formed from the internodal cell by the operation of strangulation, was also cultured in a similar manner. Even such a tied off fragment of the internodal cell showed a clear morphogenetic polarity.

4. In the culture solutions so far used, IAA-K solution was the most favorable for the growth and morphogenesis of the cell cultured in vitro. Knop's solution was next to IAA-K solution and tap water was the least favorable for the culture medium.

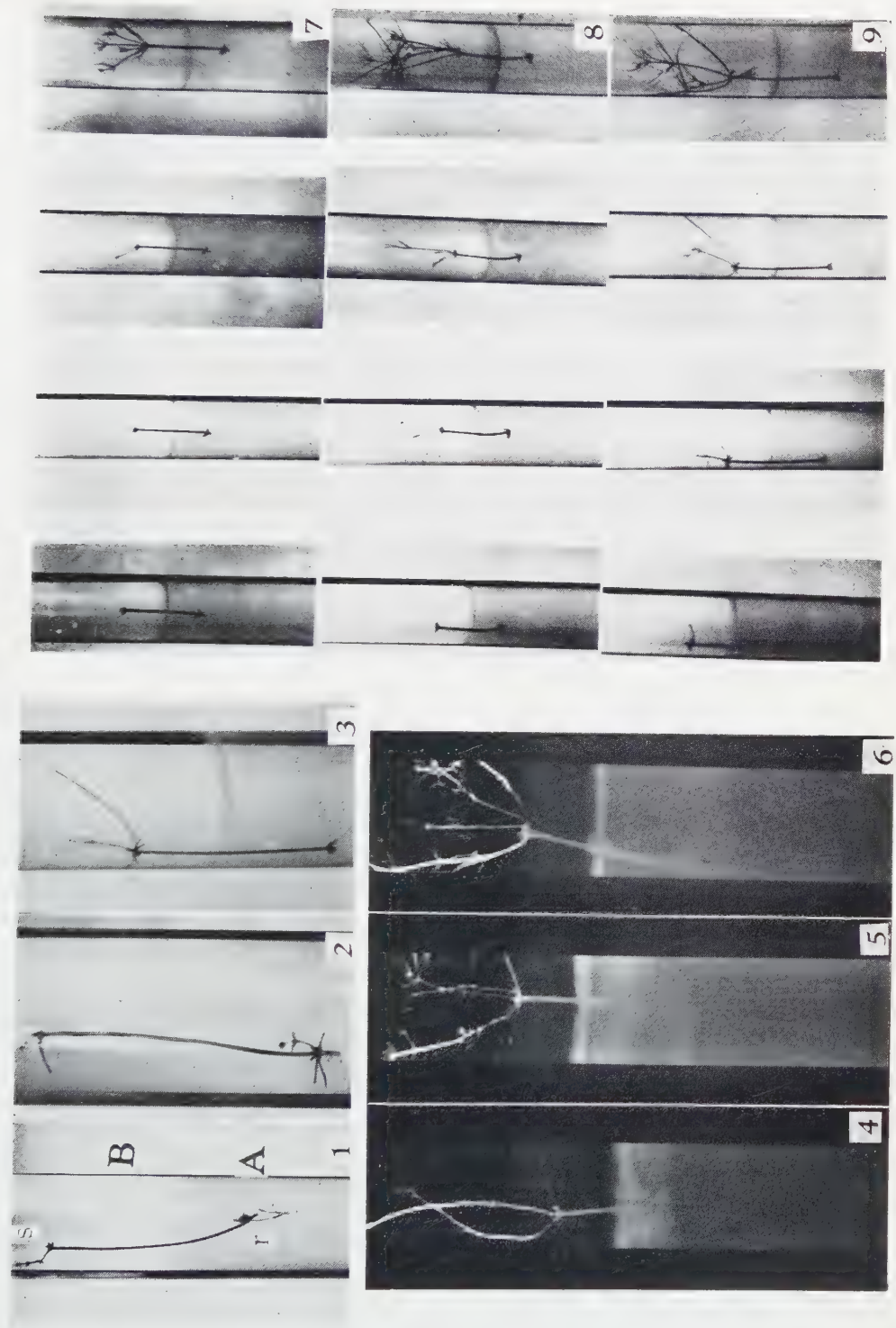
5. The rate of protoplasmic rotation in the cell cultured in vitro was nearly constant in general but it was clearly promoted during a definite period before shooting.

Literature cited

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Explanation of Figures

- Fig. 1. An isolated internodal cell cultured for 40 days in the vertical, normal position. A; agar (0.8%) region, B; tap water region, s; shoot, r; rhizoids (stained with chlorzinc iodine).
- Fig. 2. The cell cultured for 15 days in the vertical, inverted position with agar and tap water. A shoot was formed at the apical end set downward. Rhizoids were formed at the basal end but they are not obvious in this fig.
- Fig. 3. The cell cultured for 30 days in the vertical, inverted position with agar and IAA-K solution (10 mg/500 cc of d. w.). The lower half of the cell was kept dark. A shoot was formed at the basal end set upward. Rhizoids were formed at the apical end but they are not obvious in this fig.
- Fig. 4. The cell cultured for 60 days in the vertical, normal position with agar and IAA-K solution.
- Fig. 5. The cell cultured for 90 days in the vertical, normal position with agar and Knop's solution (1/20 conc. of original solution).
- Fig. 6. The cell cultured for 90 days in the vertical, inverted position with agar and IAA-K solution. The lower half of the cell was kept dark.
- Fig. 7-9. Process of shooting in the isolated internodal cell. From left to right, after 15 days, 30 days, 45 days and 60 days. Fig. 7; The cell cultured in the vertical, normal position with agar and tap water. Fig. 8; The cell cultured in the vertical, normal position with agar and IAA-K solution. Fig. 9; The cell cultured in vertical, inverted position with agar and IAA-K solution. The lower half of the cell was kept dark.
- Fig. 10. Schematic representation of the internodal cell cultured for 30 days in the horizontal position. A; tap water region, B; agar region, s; shoot, ra; rhizoids formed at the apical end, rb; rhizoids formed at the basal end.
- Fig. 11. Schematic representation of the strangulated internodal cell cultured for 30 days in the horizontal position. 1; ligature.
- Fig. 12. Relation between the rate of protoplasmic flow in the internodal cell and time. The cell was cultured in the vertical, normal position with agar and tap water.



T. SANDAN: Growth and Morphogenesis of the Internodal Cell of Characeae

クラマゴケモドキ属数種の構造的性染色体

瀬 川 道 治*

Michiharu SEGAWA: Strukturelle-Geschlechtschromosomen bei einigen Arten von *Madotheca*.

1955 年 6 月 10 日受付

先に辰野及び筆者(1955)は蘚類の一種、ツルチウチンゴケ (*Mnium Maximowiczii*) で構造的性染色体 (Strukturelle-Geschlechtschromosomen) を発見した。それは雌雄の X 及び Y が中期では、形態的の差が認められないにもかかわらず、その前期又は後期の異常凝縮において、その異常凝縮の量と分布が異っており両者の区別ができるものである。このように内部構造においてのみ異なる性染色体は、植物ではツルチウチンゴケのほかには未だ知られていないが、併しかかる例はかつて性染色体の見出されなかつた雌雄異株の蘚苔類にもありうるのではないかと考えられる。このような予想のもとに、筆者は苔類のクラマゴケモドキ属 (*Madotheca* 属) の数種について観察を行つた。けだし本属の数種では既に、辰野

(1938a, g, '41, '47) が報告している如く、雌雄何れも最大の染色体が異質染色体であるが、それが中期では形態的の差異がなく従つて性染色体として認められなかつたものである。筆者はこの研究においてクラマゴケモドキ属 6 種においても明らかに構造的性染色体を見出す事が出来たので、ここに報告する。

材料及び方法

研究に用いられたクラマゴケモドキ属 6 種の種名並びにその採集地は Tabelle 1 の如くである。固定は先ず前処理として、植物体の生長点を 0.002 mol./l の 8-oxyquinoline 水溶液中に温度 18-20°C で 2 時間保ち、その後水道水で 5 分間水洗し、これを Carnoy 液 (アルコール 3: 氷醋

Tabelle 1. クラマゴケモドキ属 6 種の種名、産地及び核型
(Artenname, Fundort und Karyotype von 6 *Madotheca*-Arten)

植 物 名 (Artenname)	核 型 (Karyotype)	産 地 (Fundort)
コクラマゴケモドキ (<i>M. densifolia</i> Stephani)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	安芸: 三段峽
トサクラマゴケモドキ (<i>M. tosana</i> Stephani)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	備後: 帝釈峽, 伯耆: 大山
クラマゴケモドキ (<i>M. perrottetiana</i> Montagne)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	三段峽, 帝釈峽
チヂミカヤゴケ (<i>M. ulophylla</i> Stephani)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	帝釈峽
ヒメクラマゴケモドキ (<i>M. setigera</i> Stephani)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	三段峽, 大山
ヤマトクラマゴケモドキ (<i>M. japonica</i> Sande Lacoste)	♀ K = V(X) + 4V + 3J ♂ K = V(Y) + 4V + 3J	三段峽

酸 1) で 1 時間固定した。染色は 2% 醋酸オルセイン液 10cc に NHCl 1cc を加えた溶液でそめ、それを 0.5% 醋酸オルセイン液におきかえて観察した。

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観 察 結 果

本研究のクラマゴケモドキ属 6 種は何れも雌雄異株である。各種の雌雄の染色体を比較した結果、6 種は核学的には殆んど同様であつたから、ここでは先ず、そのうちの一種 コクラマゴケモドキ (*Madotheca densifolia*) について主として述べる。コクラマゴケモドキの核型は先に辰野 (1941) が雌雄共に $K=V(H)+4V+3J$ とした。けだし H は最大の染色体で異質染色体 (Heterochromosom) であるが、氏はこの染色体について特に注意を払つたが、性染色体としての分化は認め得なかつた。筆者はこの H 染色体の異常凝縮の様子を詳しく観察した結果、雌雄の H 染色体が異常凝縮部分において相違する事を認め、構造的性染色体である事を知つた。即ち、雌株の X は中期核板中最大の染色体で中央に一次狭窄を有し、V 型である。そしてその一腕には少々尾部に近く明らかな二次狭窄が 1 個見られる。又他の腕には、ほぼ中央部に潜在的な二次狭窄がある (Fig. 1 c)。後期 (Fig. 1 d) になると、他の普通染色体が次第に分散して染色性を失つても、この X の大部分は異常凝縮を示して残存する。即ち、その異常凝縮部は明瞭な二次狭窄をもつ腕の尾部と潜在的な二次狭窄をもつ腕の全部である。

この時期になると、潜在的狭窄は一層明らかに認められる様になる。ついで休止期 (Fig. 1 a) においては、これらの異常凝縮部は仁の表面に接着している。即ち、X は仁染色体 (Nukleolen-Chromosomen) である。前期 (Fig. 1 b) になると、今まで分散していた染色体部分が螺旋状に現われてくるが、この際休止期に異常凝縮していた部分は分散していた部分よりも濃く染まる。

一方、雄株の Y は中期核板 (Fig. 1 g) では X との間に大いさ及び形の相違は認められない。即ち、中央に一次狭窄があり、一腕の尾部近くに明らかな二次狭窄、他腕の中央部に潜在的二次狭窄がある。唯 Y は時に一腕の中央から曲つてあたたかも J 型の如く見える事がある。後期 (Fig. 1 h) では、Y も明らかに異常凝縮を示すが、その異常凝縮部が X とは異つている。即ち、X と同様潜在的二次狭窄をもつ腕全部と他腕の尾部が異常凝縮をするほか、Y では後者の基部も異常凝縮をする。それで Y は X に比して異常凝縮部が多い。従つて、休止核 (Fig. 1 e) 中にはそれらに由来する大きな 3 個と小さな 1 個の異常凝縮があり、それが仁に接着している。そこで Y も亦、仁染色体である。前期には、休止期に分散していた部分が螺旋化して中期の染色体の形態を整え始めてくるが、この場合にも異常凝縮を示し

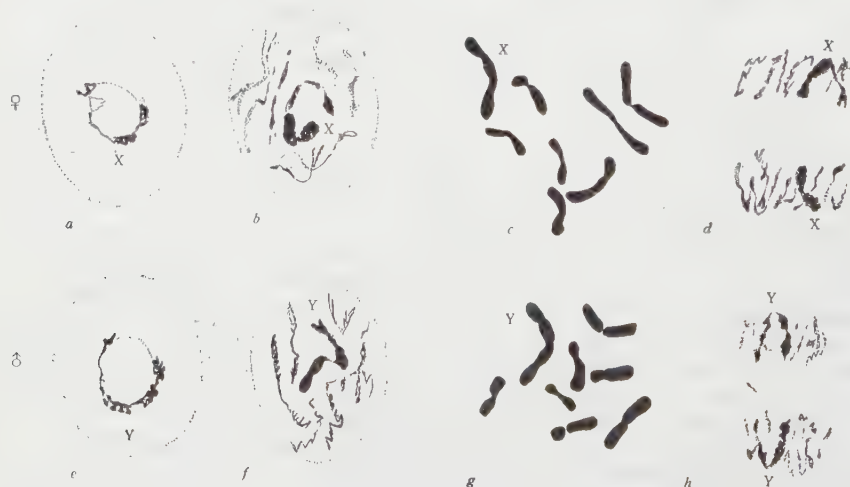


Fig. 1. コクラマゴケモドキの染色体及び異常凝縮, 配偶体

(Chromosomen und Heteropyknose von *Madotheca densifolia*, Gametophyten)

a-d ♀: a 休止核 (Ruhekern), b 前期 (Prophasekern), c 中期 (Metaphase), d 後期 (Anaphase). e-f ♂: e 休止核 (Ruhekern), f 前期 (Prophasekern), g 中期 (Metaphase), h 後期 (Anaphase).



Fig. 2. コクラマゴケモドキにおける X 及び Y の前期の変化, 配偶体

(Prophasische Veränderung der X sowie Y von *M. densifolia*, Gametophyten)

ていた部分は分散していた部分よりも濃く染まる (Fig. 1 f)。又この染色体は前期から中期にかけて、他の染色体よりも早く形態を整える様子がしばしば観察された。

Fig. 2 は更に X 及び Y の休止期, 前期及び中期における状態を互いに比較したもので、両者の相違が明瞭である。即ち、X 及び Y は中期では形態的な差異を示されないが、休止期及び前期では異常凝縮部の数と位置とが相違しており、構造的性染色体と云える。この相違は先のツルチウチンゴケの構造的性染色体 (長野, 瀬川 1955) とよく似ている。尚、性染色体以外の染色体については雌雄間に相違がみられず、本種の核型は雌雄共に $K(n)=8=V(H)+4V+3J$ である。以上の結果から本種の染色体式は、♀ $7+X$, ♂ $7+Y$ で示す事が出来る、

他のクラマゴケモドキ属 5 種即ち、トサクラマゴケモドキ (*M. tosana*) (Fig. 3), クラマゴケモドキ (*M. Perrottetiana*) (Fig. 4 a-d), チヂミカヤゴケ (*M. ulophylla*) (Fig. 4 e-h), ヒメクラマゴケモドキ (*M. setigera*) (Fig. 4 i-l) 及びヤマトクラマゴケモドキ (*M. japonica*) (Fig. 4m-p) にも上記コクラマゴケモドキと同様な構造的性染色体 X, Y が見られる。それは前種と同様、最大の V 型染色体である。即ち、中期における X 及び Y は夫々中央に一次狭

窄、一腕の尾部に二次狭窄、他腕に潜在的二次狭窄が認められ、雌雄の間に形態的の差がなく、又大いさの差もない。しかるに X 及び Y の異常凝縮はコクラマゴケモドキの場合と全く同様で、夫々コクラマゴケモドキの X, Y と同じ部分が異常凝縮を示し、従つて、Y の異常凝縮の量は常に X のそれよりも多く、構造的な相違が認められる。尚、これらの種の X 及び Y は何れも仁染色体である。因みに、ヤマトクラマゴケモドキでは中期で Y がコクラマゴケモドキの Y に見られた如く、二次狭窄のところで曲つて J 型を示すことがあつたが、他の種ではこのような事は明瞭には認められなかつた。これ等 5 種の核型も亦、前記コクラマゴケモドキのそれと同様で、雌雄共に $K(n)=8=V(H)+4V+3J$ で示される。これ等 5 種のうち、特に、トサクラマゴケモドキ (Fig. 3) 及びチヂミカヤゴケ (Fig. 4 e-h) では普通染色体のあるものにも部分的異常凝縮が認められ、しかもその異常凝縮部は休止核において、仁内に入り小仁 (Nuklolinus) を作る。既にこのような小仁を作る小仁染色体 (Nukleolen-Chromosom) は他の二、三の蘚苔類で知られて、ケゼニゴケ (*Dumortiera*



Fig. 3. トサクラマゴケモドキの染色体と異常凝縮, 配偶体
Chromosomen und Heteropyknose von *M. tosana*, Gametophyten)

a-c ♀: a 休止核 (Ruhekern), b 前期 (Prophasekern), c 中期 (Metaphase).
d-f ♂: d 休止核 (Ruhekern), e 前期 (Prophasekern), f 中期 (Metaphase).

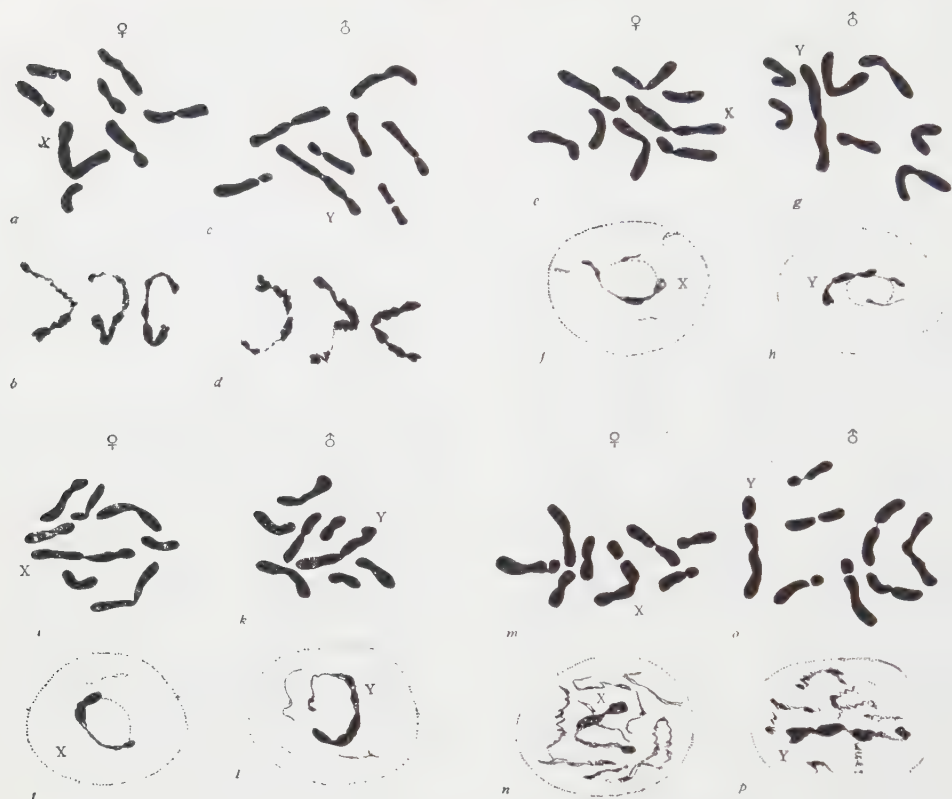


Fig. 4. クラマゴケモドキ 4 種の染色体と異常凝縮, 配偶体

(Chromosomen und Heteropyknose der vier *Madotheca*-Arten, Gametophyten)

a-d, クラマゴケモドキ (*M. Perrottetiana*)

a, b ♀: a 中期 (Metaphase), b X の前期の変化 (Prophasische Veränderung der X)

c, d ♂: c 中期 (Metaphase), d Y の前期の変化 (Prophasische Veränderung der Y)

e-h, チヂミカヤゴケ (*M. ulophylla*)

e, f ♀: e 中期 (Metaphase), f 休止核 (Ruhekern)

g, h ♂: g 中期 (Metaphase), h 休止核 (Ruhekern)

i-l, ヒメクラマゴケモドキ (*M. setigera*)

i, j ♀: i 中期 (Metaphase), j 休止核 (Ruhekern)

k, l ♂: k 中期 (Metaphase), l 休止核 (Ruhekern)

m-p, ヤマトクラマゴケモドキ (*M. japonica*)

m, n ♀: m 中期 (Metaphase), n 前期 (Prophasekern)

o, p ♂: o 中期 (Metaphase), p 前期 (Prophasekern)

hirsuta, 辰野 1954 a, b), ツルチヨウチンゴケ (*Mnium Maximowiczii*, 辰野, 瀬川 1955) では異常凝縮性の m 染色体, ミドリミズゼンゴケ (*Riccardia pinguis*, 辰野, 瀬川 1955) では附随体染色体の異常凝縮性の染色体であつた。筆者の観察したクラマゴケモドキ属の種には m 染色体がないから, これ等2種の小仁は何れの染色体に起因するか今後の研究によつて明らかにしたい。

以上のべた如く, これ等5種はコクラマゴケモドキ同様, 夫々構造的性染色体をもつ事が認められ, その染色体式は何れも ♀ 7+X, ♂ 7+Y である。

構造的性染色体の例は植物においては藓類のツルチヨウチンゴケについて発見されたものであり, 苔類においてはこれ等6種が最初の例である。思うに, これ等の構造的性染色体は藓苔類の性染色

体の進化過程からみて、形質的に相違する性染色体へ分化する基本型としての意義をもつものではないだろうか。

もとに行われたものであり、材料の採集、同定にあつては安藤久次氏の援助をうけ、研究の遂行にあつては下斗米直昌教授から教示を賜つた事を附記して諸先生に対し感謝の意を表わすものである。

終りに臨み、この研究は長野誠久博士の指導の

Résumé

Um die Geschlechtschromosomen genau zu erkennen, habe ich in dieser Abhandlung 6 diözische *Madotheca*-Arten, nämlich *M. densifolia*, *M. tosana*, *M. Perrottetiana*, *M. ulophylla*, *M. setigera* und *M. japonica*, untersucht.

Bei dieser 6 Arten konnte ich zum ersten Male die Strukturelle-Geschlechtschromosomen entdecken. Die Chromosomenformeln dieser Pflanzen sind die folgenden;

	♀	♂
<i>M. densifolia</i>	7+X	7+Y
<i>M. tosana</i>	7+X	7+Y
<i>M. Perrottetiana</i>	7+X	7+Y
<i>M. ulophylla</i>	7+X	7+Y
<i>M. setigera</i>	7+X	7+Y
<i>M. japonica</i>	7+X	7+Y

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酵母の葡萄糖の酸化ならびに醗酵に対するデフォス フォピリジンヌクレオチドの影響について

西 上 一 義*

Kazuyoshi NISHIGAMI: Effect of Diphosphopyridine Nucleotide on Oxidation
and Fermentation of Glucose by Yeast

1955 年 7 月 17 日受付

Meyerhof は 1920 年代の研究¹⁾で、野性酵母、パン酵母、上面ビール酵母、下面ビール酵母等の QO_2 , $Q\overset{N}{CO}_2$ を測定した。その結果によると、酵母の呼吸能は、酵母の原体と見られる野性酵母から、パン酵母、上面ビール酵母、下面ビール酵母の順に急激に減少している。これに平行して醗酵能も大体この順序でわずかに減少している。これらの酵母について、 $Q\overset{N}{CO}_2$ と QO_2 との比 ($Q\overset{N}{CO}_2/QO_2$) を出すと、その値は、野性酵母、パン酵母、上面ビール酵母、下面ビール酵母の順に増加してくる。これは人為的な培養条件によつて、野性酵母から変化して来た結果と考えられており、勿論この ($Q\overset{N}{CO}_2/QO_2$) の値の増加するに従つて、エネルギーの獲得については、呼吸より醗酵に対する依存度が大きくなって来る。以上のような従来見られた醗酵と呼吸との相互変化の現象が、生体内でいかなるメカニズムで行われ、酵素系にどのような変化がもたらされているかを知ることが、比較生化学の立場から重要であると考へ、以下にのべるような実験を行つた。

材料及び方法

材料は、下面ビール酵母として、北海道大学農学部応用菌学教室保存の *Saccharomyces carlsbergensis* (S 28) を、パン酵母は日本甜菜糖株式会社のものを用いた。

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培養は Henneberg 氏液を用い、これに 0.5% Yeast extract を加えた。その組成は次の通りである。

蒸留水 1000 cc, 蔗糖 150 g, プロトン 5 g, KH_2PO_4 5 g, $MgSO_4$ 2 g, Yeast extract 0.5%, pH 5.4, 温度 24°C, 培養時間 48 時間, 250 cc 三角コルベン中に培養した。これを乾燥酵母にして使用した。

乾燥酵母は、48 時間培養した酵母を遠心分離器を用いて蒸留水で 3 回洗い、布でしぼつて水分を除き、それをシャーレの上に薄く延ばして、真空デシケーター中で出来るだけ早く乾燥させたもので、乾燥後、乳鉢で細粉にして用いた。

DPN は Euler²⁾の方法によつて作製した。

QO_2 , $Q\overset{N}{CO}_2$ は Warburg 検圧計によつて測定した。温度は 30°C, 反応液の組成は次の通りである。M/5 葡萄糖 0.5 cc, M/5 pH 5.4 磷酸緩衝液 0.5 cc, DPN 2mg/cc 0.5 cc, 酵母浮遊液 0.5 cc, を主室に入れ、副室に QO_2 測定の際は 20% KOH 0.5 cc, $Q\overset{N}{CO}_2$ 測定の際は蒸留水 0.5 cc を入れて、合計 2.5 cc とする。

この実験では葡萄糖を基質として与えた時に、DPN を加えた場合と加えない場合の QO_2 差から、葡萄糖の代謝系中、DPN が関与する酵素の量及びこの酵素の蛋白部分と助酵素との量的相互関係を比較した。これと別に、アルコール脱水酵素の活性をツンベルグ管によるメチレンブルー脱色の方法によつて測定した。また CO_2 の発生量をワールブルグ検圧計によつて測定し、それに対する DPN の影響をしらべた。

実験結果

第1表

2種の酵母に基質として葡萄糖を与えた際の、 Q_{O_2} と $Q_{CO_2}^{N_3}$ 及びそれに対する DPN の影響

パン酵母				ビール酵母			
Q_{O_2}		$Q_{CO_2}^{N_3}$		Q_{O_2}		$Q_{CO_2}^{N_3}$	
DPN		DPN		DPN		DPN	
-	+	-	+	-	+	-	+
0.33	0.62	4.70	15.3	0.26	0.40	1.40	1.50
0.62 = 1.9 0.33		15.3 = 3.3 4.70		0.40 = 1.5 0.26		1.5 = 1.1 1.4	

第1表に示すように、パン酵母では、DPN を与えることによつて、葡萄糖の Q_{O_2} も $Q_{CO_2}^{N_3}$ も共に増加するが、ビール酵母では Q_{O_2} のみ増加して、 $Q_{CO_2}^{N_3}$ に対しては殆ど影響が見られなかつた。 Q_{O_2} 増加の度合はパン酵母の方が大きかつた。

第2表

2種の酵母に基質としてエタノールを与えた際の、メチレンブルー脱色時間及び DPN の影響

Ethanol	-			+
DPN	-	-	-	+
パン酵母	>360	25	6	
ビール酵母	>360	55	10	

DPN 1mg, 乾燥酵母 10mg, M/6 エタノール 1 cc, M/1600 メチレンブルー 0.5 cc, 蒸留水 3.5 cc, pH 5.4, 温度 30°C, 単位は分で表わした。

第2表に示す実験によつて、2種の酵母が共に DPN を添加することによつて、エタノールを基質としてのメチレンブルー脱色の時間が短縮するのが見られた。この場合ビール酵母の方が効果はいちぢるしかつた。

結 論

酵母の中ではパン酵母の方がビール酵母に比べ

て、葡萄糖の酸化能も無酸素的分解能も強いということはすでに知られていることであるが、葡萄糖分解の複雑な管路の中で、どの部分反応にあづかる酵素系に差があるかについての詳細はまだ明らかになつていない。

この問題の解明には、葡萄糖分解管路の各部分反応に関与する酵素についてその活性を比較しなければならないが、こゝではとりあえず酵母細胞全体を乾燥酵母の状態にして透過性を増大させたものを使用し、その呼吸及び醗酵に対する DPN 添加の影響をしらべてみた。実験の結果明らかになつたことは DPN 添加の効果が呼吸の場合にも、醗酵の場合にもビール酵母に比べてパン酵母の方がいちぢるしいということであつた。このことは、酵母乾燥の際における DPN の不活性化等による成分の割合が、一定両酵母について等しいと仮定すれば、パン酵母の方がビール酵母に比べて DPN の欠乏の状態にあること、いふかえれば、パン酵母においてはビール酵母に比べて、呼吸ならびに醗酵の機作に関与する酵素系の中で DPN を助酵素とする酵素の蛋白部分が、助酵素に比して過剰であることを示している。

葡萄糖の無氧的分解は酵母においては典型的な Embden-Meyerhof-Parnass 図式に従うものと考えられるから、この図式中で DPN が関与する系は、グリセリンアルデヒド脱水素酵素とアルコール脱水素酵素である。ビール酵母の呼吸ならびに醗酵がパン酵母に比べて弱いことは、葡萄糖の代謝にあづかる酵素系の蛋白部分の生体内合成能が、パン酵母に比べてより退化しているからである。

メチレンブルー脱色実験によるアルコール脱水素酵素の活性もビール酵母の方がパン酵母より弱い。しかし DPN 添加による脱色時間短縮の割合は、この場合にはビール酵母の方がいちぢるしかつた。細胞中の DPN の含量はビール酵母の方がパン酵母より高いことが知られているが³⁾、アルコール脱水素反応に関してはビール酵母はなお DPN の不足の状態にあるように考えられる。

要 約

- 1. パン酵母はビール酵母より呼吸及び醗酵が強い。
- 2. DPN を与えると呼吸及び醗酵が促進され

るから、その場合はパン酵母の方がいちじるしい。

効果はビール酵母の方がいちじるしい。

3. アルコール脱水素能に対する DPN 添加の

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Résumé

1. The effects of DPN-addition on the oxidation and anaerobic fermentation of glucose were compared between bakers' yeast and brewery yeast.
 2. Bakers' yeast respire and ferments glucose more intensively than brewery yeast. Stronger acceleration of both respiration and fermentation by DPN addition was observed in the case of bakers' yeast compared with brewery yeast.
 3. Smaller respiratory and fermentative activity of brewery yeast seem to be ascribed to low contents of enzyme proteins in the yeast cells.
 4. The intensity of alcoholic dehydrogenase is greater in brewery yeast cells than in bakers' yeast cells. DPN-deficiency is also greater in brewery yeast.
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Cytological and Morphological Studies on the Gametophytes of Ferns IX

The Polar Plasmolysis on Fern-prothallium (4)

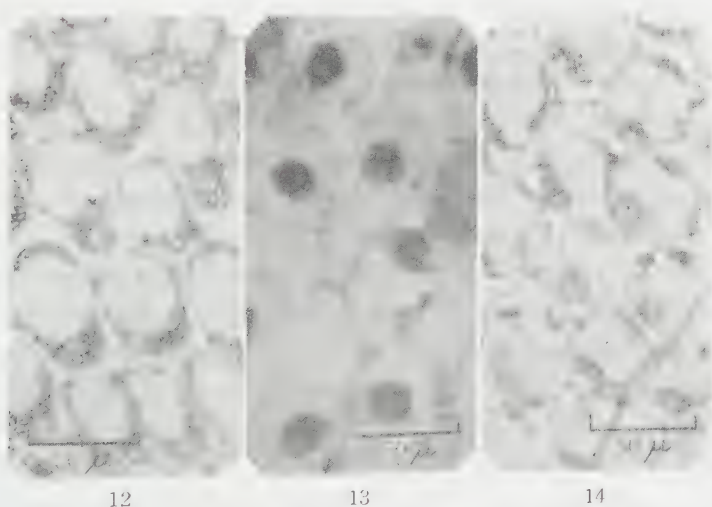
by Isami IGURA*

伊倉伊三美：羊齒類の配偶体に関する細胞学的並に形態学的研究 IX
羊齒類前葉体の有極性原形質分離 (4)

February 2, 1955

V. Behaviour of the plastid

The behaviour of the plastids was observed with interest in plasmolysis or deplasmolysis in many cases. When the prothallial cells of *Asplenium incisum* were plasmolysed in KNO_3 - (e.g. 0.3 mol.), KCl - (e.g. 0.9 mol.), NaCl -, AlCl_3 -, MgCl_2 -, and glycerin-solutions, the special behaviour of the plastids was recognized. The



Polar plasmolysis of the prothallial cell in *Asplenium incisum* Thunberg.

Photos. 12, 13. Plasmolysis in 0.52 mol. NaCl -solution ("Systrophe"). 12. Region IV; after sixty minutes. Plastids are moving along the membrane towards the basal pole. 13. Region III; after about twenty-four hours. Cytoplasm shows the perfect round A-type and the plastids adhere closely together in a round lump surrounding the nucleus.

Photo. 14. Deplasmolysis in 1.0 mol. urea-solution after seventy minutes. Region III. Plastids are moving along the membrane from the region of the apical pole towards that of the basal one as in Photo. 12.

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plastids diffused in the cell began to move along the cell wall from the apical pole towards the basal pole, when the form of plasmolysis gained A-type after about forty minutes to one hour. And after about two hours or more, the plastids clustered at the basal pole and then they adhered closely together in a round lump, perhaps surrounding the nucleus inside the plasmolysed cytoplasm of the cell after about twenty four hours (Photos. 12, 13). In the deplasmolysis caused by urea-solution (e.g. 0.4, 0.6, 0.7, 0.8 and 1.0 mol.) the same behaviour of plastids was observed (Photo. 14). This phenomenon is said to be the "Systrophe" which was reported by Germ^{5,6,7)} in other plant cell and the present writer was able to find this fact in the cell of the fern-prothallium as reported already in the previous paper¹⁰⁾. Sometimes the clustered plastids diffuse again inside the cytoplasm (0.8 mol. urea-solution, deplasmolysis).

Discussion and Conclusion

The present experiments were carried on mainly concerning the adult prothallia, grown up in the thermostat or the laboratory room for about one year and a half, receiving no special influences of the external conditions as stated above, so no external factors will be noticed here. In the whole prothallium, some gradients, that is, the cyto-physiological gradients are found in the form and duration of plasmolysis, in osmotic value, in deplasmolysis, and so on. These gradients, the author assumes, are due to the orientation of the growth and development in the whole prothallium and so are the polarity of the prothallium, and it is suitable to classify the field of the prothallium into the following three polarities: that is, the longitudinal ("medial" of Reuter), the tangential, and the radial. Reuter¹⁸⁾ reported also the polarities like these. Esterřák³⁾ reported the "Grundgradienten" which are caused by the physical and chemical conditions in the leaf-cells of *Elodea*. In 1938, Drawert¹⁾ proved the gradients of stainability in the *Helodea*-leaves (*H. canadensis*, *H. densa*) fixed with 70% alcohol, and explained that the isoelectric points of the different parts of cells are related to the degrees of differentiation of the cells. Moreover, he made clear the following facts. The smaller the IEP, the younger the cell membrane was, and this phenomenon might be due to the abundant contents of protein substance in the young cell membrane. The other cellular elements seem to show generally the greater IEP in the young cells than in the adult ones.

The plasmolyses of the prothallial cells revealed the gradients in the longitudinal polarity distinctly, and in other polarities also the gradient seemed to be observed. Therefore, the writer terms tentatively the apical portion of the fern-prothallium as apical pole (meristem-pole), whose cells are young and capable of dividing, while the basal portion as basal pole (protonema-pole), whose cells are older than those of the meristem and form the filamentous protonema and its neighbourhood. In a single prothallial cell the polarity is also considered to be recognized. The cyto-physiological polarity of the single cell may naturally be controlled by the polarity

of the whole prothallium, and considered to arise by reason of the inner differentiation of cytoplasm in the cell, or the physico-chemical or the chemical nonhomogeneous structure. That is to say, the viscosity of cytoplasm, the contacting power of cytoplasm to the cell wall, and the permeability for water or reagents are in the state of inequality.

The forms of plasmolyses are different at the basal pole and the apical one in the same concentration of the same reagent during the same time, and while the grade of the plasmolysis reaches the maximum at Region I or II, it decreases more and more according as the region approaches Region VI. Consequently, the duration of the plasmolysis is minimum at the basal pole and maximum at the apical one.

The osmotic value is smallest at the basal pole and increases gradually towards the apical pole, so it is largest at Region VI, and the increasing ratio grows large as the region draws near the Region VI. It is said that the results of Gratzy-Wardengg⁸⁾ gave a foundation to the correct experiments of the author. As to the tangential polarity or the radial also, we may be able to affirm the same relationship between the basal and the apical pole. From these points of view, the following conclusion can be drawn out. Namely, the permeability of the cytoplasm to various reagents is lower at the basal pole than at the apical one, and the viscosity, too, is smaller at the former than at the latter, so the permeability or the viscosity increases by degrees as the region comes near the apex. In many cases, really it is observed that the incipient plasmolysis occurs first at the apical pole of the prothallial cell.

Comparison of the osmotic values of urea and glycerin is made in the present experiment and the characterized difference between the basal and the apical pole was obtained. Urea is well known reagent which possesses high permeability, and in its solution the deplasmolysis of the prothallial cell occurred clearly and the duration of deplasmolysis is short. Both in urea- and glycerin-solutions the plasmolysis begins at the basal pole and extends towards the apical; on the contrary, the deplasmolysis begins at the latter and extends towards the former in the whole field of the prothallium. Therefore, we know that urea or glycerin is more permeable to the apical cell than to the basal one. Presuming from the results shown in Fig. 16, glycerin is more permeable than urea to the cell of apical region whose permeability is to be called the glycerin-type, while less permeable than urea to the cell of basal region whose permeability the urea-type.

The isotonic coefficient (i) is greatest at the basal pole and tends to decrease gradually as the region comes near the apical pole. Comparing the isotonic coefficients of the electrolytes with the coefficients of van't Hoff physico-chemically known or with Fitting's results⁴⁾, we find the approximate agreement in the basal region especially in CaCl_2 -, KNO_3 -, and KCl -solutions, though the considerable difference is recognized in Na_2SO_4 -solution.

The permeability coefficient (μ) is smallest at the basal pole and is apt to increase more and more as the region draws near the apical one. This fact confirms

the cells at the protonema portion possess the lowest permeability and the meristem portion the highest.

The apparent osmotic values of fern-prothallium which were determined with electrolytic reagents are smaller than those determined with the nonelectrolytic ones. The ion series of the former is shown as follows: $Mg > Al > NH_4 > Na > Ca$, K , and the series of the latter is as follows: urea $>$ glycerin $>$ glucose $>$ saccharose. The permeability coefficient of some reagent, i. e. $CaCl_2$, KCl , KNO_3 , and $NaCl$, is negative. In the present experiment, this fact may be considered that these reagents are less permeable to the cytoplasm as ion or molecule than saccharose. The behaviour of the plastid in plasmolysis or deplasmolysis is considered to be the phenomenon known as "Systrophe" of Germ^{5,6,7}, and the plastids diffused in the cytoplasm gathered finally in a round lump at the basal pole of the cell. This phenomenon is presumed to be caused by the decrease of the viscosity in the cytoplasm. The existence of the polarity in the whole field of the fern-prothallium may be related to the polarity in the spore germination or the plasm differentiation in a single prothallial cell, and then as to the problem what factors determine finally the polarity of the whole prothallium, further consideration may be needed for us.

Summary

Carrying out the osmotic experiments for the adult prothallia of *Asplenium incisum* Thunberg and other three species by the use of the reagents of urea and other fourteen kinds as the plasmolytica, the writer obtained the following results.

1. The cyto-physiological gradients are demonstrated in the form and duration of plasmolysis, the permeability, the osmotic value, the isotonic and the permeability coefficient, or the deplasmolysis in the cells of fern-prothallium.

2. The existence of polarities is found in the whole field of the prothallium and also in a single prothallial cell. Concerning the polarity, the writer termed the basal region of the protonema as a basal pole (protonema-pole) and the apical region of the meristem as an apical pole (meristem-pole).

3. Although the form and duration of plasmolysis are changeable in the same plasmolyticum and mol.-solution according to the length of the treatment, the plasmolysis occurs earlier at the basal pole than at the apical one and it extends gradually from the former towards the latter.

4. The permeabilities of urea and glycerin are considerably high to the prothallial cell, whereas the former is more permeable than the latter at the basal region (urea-type) and less than the latter at the apical one (glycerin-type).

5. The osmotic value and permeability coefficient are lowest at the basal pole and are apt to increase approaching the meristem where the highest value is found. The isotonic coefficient appears to show the reverse result against the permeability coefficient.

6. The plastids in the prothallial cell behave themselves as if they indicated

the polarity of the cell and reveal the phenomenon of "Systrophe", clustering in a round lump at the basal pole.

The present author expresses his hearty gratitudes to Prof. Dr. A. Yuasa and Prof. Dr. H. Ito for their valuable instructions and influential criticisms in this study, and also to Prof. Dr. T. Miwa for his valuable guidances and very helpful suggestions.

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A Study of Tropism of Pollen Tubes to Pistils II Tropism in *Camellia sinensis*

by Hisako MIKI*

三木寿子: 花粉管のシズイにおける屈向性 II. *Camellia sinensis* の屈向性

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When pollen grains are spread around pistil slices put on an agar culture medium, the pollen tubes are attracted to the sliced pieces. This phenomenon was observed by Molish (1894), Miyoshi (1894), Lidforss (1909), Brink (1924), Tsao (1949) and Iwanami (1953) in some higher plants. Among these investigators, while Miyoshi found sucrose caused positive tropism of pollen tubes, Tsao was not able to find any special substance to which pollen tubes showed positive tropism. The present author has reported that an active substance, which is responsible for the positive tropism of pollen tubes to pistils, is present in some tissues of the pistils of *Lilium longiflorum* and *L. japonicum* (Miki, 1954).

In the above and the following investigations, the author intends to see whether this special substance is found in pistils of other higher plants or not, and to study the rôle of this substance in the course of fertilization. In the present investigation it is intended to study the special substance in *Camellia sinensis*.

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Material and Method

Pollen grains and pistils of *Camellia sinensis* known for its long flowering season, were used as material.

For culture media of pollen grains, 1.5% agar soln. containing 10% sucrose was employed. The hydrogen ion concentration of these media was between pH 6.2 and 6.4.

The sugar-agar solution was put on a slide glass to form a layer about 2mm in thickness, and when it had solidified, slices of pistils were put on the agar plate. Around these slices pollen grains were spread with a slender brush. Then the slide glass was placed in a Petri dish and was kept in an incubator at 30°C. After 2-3 hours, pollen grains which were put within 1mm from the slices were examined. The number of total pollen grains on each slide was about 200-500.

In control slides, in place of a pistil slice, a stripe was marked or a quartz-sand was put on the agar film.

Grades of germination and tropism are presented in this paper by “percentage of germination”^{**} and “percentage of tropism”^{***} respectively.

Experiments

1. Tropism of pollen tubes to styles and ovaries

A pistil was cut into two parts, a style and an ovary. Then each part was cut longitudinally, followed by a transversal cutting in 3-4 mm in length. These slices were put on the agar film so that the longitudinal plane of the slices was in contact with the surface of the film. Pollen grains were spread with a brush around these slices. After 2 or 3 hours, behavior of pollen tubes was examined.

In this experiment it is observed that pollen tubes elongate smoothly to the slices of pistils as if the tubes were attracted to the slices. Tropism of this type is designated as “positive tropism” in this paper. Results obtained in this experiment are shown in Table 1.

Table 1. Percentage of positive tropism of pollen tubes to pistil slices

Materials	Style	Ovary**	Stripe (Control)	“Quartz-sand” (Control)
tropism	96	24	30	20

* Percentage (%) of germination = $\frac{\text{Number of germinated pollen grains}}{\text{Number of total pollen grains}} \times 100$

** Percentage (%) of tropism = $\frac{\text{Number of pollen tubes that reached pistil slices}}{\text{Number of germinated pollen grains}} \times 100$

*** It must be noted here that the pollen grains around the slices of style show a high percentage of the germination and a good tube growth, while around the slices of ovary, show a low percentage of the germination and a bad tube growth. Therefore, in the following test, the ovary part was excepted.

From this table, it is seen that pollen tubes show positive tropism to the styles, but do not show to the ovaries.

Same experiments were carried out in *Camellia japonica* and *C. Sasanqua*. In these plants pollen tubes showed positive tropism to the style as observed in *C. sinensis*.

2. Tropism of pollen tubes to steamed pistils

a. Tropism of pollen tubes to steamed slices of styles and ovaries. Test tubes with pistils and moist filter papers were kept in boiling water (99°C) for 10 minutes. Then the pistils were cut and tested as in Experiment 1.

In this Experiment 2, it is observed that pollen tubes elongate as in Experiment 1. The pollen tubes which are far from the slices elongate quite at random in respect to direction, but when these pollen tubes approach to the steamed slices of the styles at the distance of 0.2-1 mm from the slices, most of the tubes change the direction and elongate in opposite from the steamed slices. Pollen tubes which are near the slices elongate in the reverse direction of the slices (see Fig. 1). Tropism of this type is designated as "negative tropism" in this paper. Results obtained are given in Table 2.

As shown in Table 2, all pollen tubes do not shown positive tropism but negative tropism (Fig. 1). From this result, it is assumed that some changes which cause negative tropism of pollen tubes have taken place in the styles by steaming.

b. Effect of steaming on tropism. From the results of the Experiment a, it is found that pollen tubes show negative tropism to the steamed styles, while they show positive tropism to fresh styles. It is intended, in this Experiment b, to see the effects of high temperature as well as time of steaming on the styles.

(1) Time of steaming Pistils were steamed in a test tube for 3, 5, 10 and 20 minutes respectively, and then the styles were cut and tested. The results obtained in this experiment are shown in Table 3.

In this table, it is seen that the percentage of positive tropism is remarkably low and most pollen tubes show negative tropism to the styles steamed for 3 minutes. Contrary to the above case, all pollen tubes show negative tropism when the styles are steamed for 5, 10 and 20 minutes.

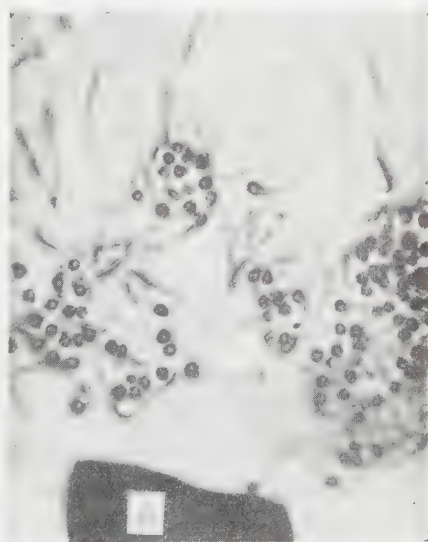


Fig. 1. Negative tropism of pollen tubes to steamed style slices in *Camellia sinensis* (steamed for 10 minutes at 99°C)

A: A steamed style slice.

Table 2. Percentage of tropism of pollen tubes to steamed pistils

Materials	Style	Ovary	Control
positive tropism	0	0	30
negative tropism	100	100	0

Table 3. Percentage of tropism of pollen tubes to styles steamed in several periods of time

Time of steaming in min.	0	3	5	10	20	Control
positive tropism	70	11	0	0	0	30
negative tropism	0	80	100	100	100	0
germination	100	100	100	100	100	90

(2) Temperature of steaming. Pistils were kept in test tubes at 15°C (room temperature), 30°C, 40°C, 50°C, 60°C, 80°C and 99°C for 10 minutes. The steamed styles were cut and tested as in the case of Exp. 1. The results obtained in this experiment are shown in Table 4.

Table 4. Percentage of tropism of pollen tubes to styles steamed at several temperatures

Steamed temperature	15°C	30°C	40°C	50°C	60°C	80°C	99°C	Control
positive tropism	83	76	69	66	10	0	0	30
negative tropism	0	0	0	0	70	100	100	0
germination	100	100	100	100	100	100	100	90

In this experiment it is observed that majority of pollen tubes treated at 15°C, 30°C, 40°C and 50°C show positive tropism while all tubes steamed at 80°C and 99°C show negative tropism. It is highly probable that an active substance responsible for positive tropism is decomposed at 60°C or thereabouts.

3. *Diffusion of active substance from styles to agar media*

Slices of fresh or steamed styles were placed on the surface of agar films on slide glasses, and were kept for 1/2, 1 or 2 hours in a Petri dish with a piece of moist filter paper. Then the slices were removed immediately after the spreading of pollen grains around the slices (and examined as in Experiment 1).

In this experiment, it is found that the tubes show positive or negative tropism to the place on agar media where the slices had been placed. The result obtained in this experiment is given in Table 5.

Results of this experiment show that the active substance, which is responsible for positive or negative tropism, diffuses from style slices to the agar media within 1 hour, but the percentage of positive tropism is lower than that obtained in Experiment 1.

Table 5. Percentage of tropism of pollen tubes to place occupied by steamed styles

Styles were placed on agar film for		1/2 hr.	1 hr.	2 hrs.	Control
Fresh styles	positive tropism	15	48	56	30
	negative tropism	0	0	0	0
Steamed styles	positive tropism	0	0	0	
	negative tropism	100	100	100	

4. Diffusion of active substance through collodion membrane

In this experiment, fresh or steamed style slices were wrapped in a collodion membrane* and put on the agar media. Then, pollen grains were spread around these wrapped styles. Reaction of tubes to the wrapped styles were examined as in Experiment 1. Some control tests were carried out with unwrapped style slices and collodion membrane without the slices. The result obtained in this experiment is shown in the following table.

Table 6. Percentage of tropism of pollen tubes to style slices wrapped in collodion membrane

Objects		Wrapped in membrane	Membrane without style	Unwrapped style
Fresh styles	positive tropism	67	27	76
	negative tropism	0	0	0
Steamed styles	positive tropism	29	27	0
	negative tropism	0	0	100

From this table, it is seen that the substance which is responsible for the positive tropism diffuses through the collodion membrane while the substance which causes negative tropism does not diffuse through the membrane. This fact suggests that the molecular weight of the former substance is lower than the latter.

Considering from the result obtained in the preceeding experiments, it is highly probable that a chemical substance which is responsible for the positive or the negative tropism of pollen tubes is contained in pistil tissues. The separation of these active substances from styles is under investigation.

Conclusion

In *Camellia sinensis*, the pollen tubes show positive tropism to the fresh pistil slices on the agar media. From the result of the present investigation, it is concluded that positive tropism of pollen tubes is caused by an active factor contained in the pistil tissues. This active factor diffuses from pistil slices to agar media and

* Congo-red did not pass the membrane within 24 hours.

diffuses through a collodion membrane. It is highly probable, therefore, that the active factor is a chemical substance with relatively low molecular weight though this substance has not been separated in pure state in this experiment.

Contrary to the above case, the pollen tubes show negative tropism to steamed pistil slices, and it is concluded that the negative tropism of the pollen tubes is caused by an active substance. This substance diffuses from steamed pistil slices to agar media, but does not diffuse through the collodion membrane. It is extracted with distilled water, and seems to have a higher molecular weight than the substance which is responsible for the positive tropism.*

The behavior of pollen tubes to the steamed pistils, stated above, is different in various plants. For example, while Tsao (1949) has reported in *Hippeastrum Johnsoni* of positive tropism of the pollen tubes to steamed pistils taking place, the present author has confirmed that the pollen tubes of *Camellia sinensis* show negative tropism to the steamed pistils. In *Lilium longiflorum* and *L. japonicum*, however, it was found that the steamed pistil tissues gave no effect on the behavior of the pollen tubes (Miki, 1954)*. As can be seen from above, the tropism of pollen tubes to pistil tissues seems to be a very complex phenomenon, but it may be stated that some chemical substances play an important rôle in this phenomenon though the nature of these substances is not cleared in the present investigation.

Summary

1. In *Camellia sinensis*, pollen tubes show positive tropism to the fresh style slices while they show negative tropism to the slices of the styles which are steamed for 10 minutes at 60°C, 80°C or 99°C.

2. Both substances, which are responsible for the positive and the negative tropism, diffuse from styles to agar media within ca. 1 hour, and the former may diffuse through a collodion membrane, while the latter does not diffuse through that membrane.

This author takes pleasure in expressing her sincere appreciation for helpful suggestions throughout this work to Professor N. Sinke and Mr. K. Katô of the Kyoto University.

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* To be published later.

* According to a verbal information of Prof. Imamura, that the pollen tubes of *Lilium philippinensis* are observed to show negative tropism to the fresh pistil slices of *Crocus sativus*.

Studien über Anthocyane XXVII¹⁾

Papierchromatographische Übersicht der Anthocyane im Pflanzenreich (II)²⁾. Farbstoffe des roten Herbstlaubes

von Kôzô HAYASHI und Yukihide ABE*

林 孝三・阿部幸願: アントチアン色素の研究 (第 27 報), 紅葉の色素

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Seitdem Chrysanthemin von Sh. Hattori und K. Hayashi¹⁾ als einen in der roten Farbe von den Herbstblättern einiger Ahorn-Arten verantwortlichen Farbstoff isoliert wurde, sind Untersuchungen über das Herbstrot auch in anderen Ländern unternommen worden. Vorerst seien angegeben die umfangreichen Arbeiten von Robinson und Robinson²⁾ über die Herbstströte englischer Pflanzen und von Lawrence u. A.³⁾, ferner jene von Price und Sturgess⁴⁾, welche letztere ausschliesslich auf der von Robinson und Robinson ausgearbeiteten qualitativen Untersuchungsmethode basierte. Neuerdings hat auch H. Reznik⁵⁾ einen vorläufigen kurzen Bericht über die Farbstoffe von deutschen herbstlich roten Blättern veröffentlicht.

In der vorliegenden Mitteilung möchten wir die Verbreitung der Anthocyane in den herbstlichen Blättern von japanischen Pflanzen, insbesondere der Bäume diskutieren.

Es wurden herbstlich rote Blätter von 74 Pflanzenarten aus 25 Familien auf papierchromatographischem Wege untersucht. Die Ergebnisse haben gezeigt, dass hierbei das Anthocyan hauptsächlich aus einem Cyanidin-monoglykosid, vorzugsweise Chrysanthemin, besteht. Dieses Ergebnis stimmt mit den früheren Befunden überein^{1 5)}. Also liegt der Schluss nahe, dass in den Blättern eine metabolische Tätigkeit vorherrsche, die das Zusammenwirken von Brenzcatechin- und Phloroglucin-abkömmlingen zur Bildung von Cyanidin-Derivaten erlaubt.

Hinsichtlich der Lokalisation des Anthocyans im Blattgewebe ist es von Interesse, dass in der Blattepidermis im Gegensatz zu den Blütenblättern eine metabolische Aktivität bezüglich der Anthocyan-synthese in der Regel nicht vorhanden zu sein scheint.

An dieser Stelle sprechen wir Herrn Dr. K. Oguma, Direktor des Nationalen Instituts für Genetik, und Herrn Dr. Y. Asahina, Direktor der Forschungsanstalt für Naturerzeugnisse, ferner auch Dr. Sh. Hattori, Professor der Tokyo Universität, unseren tiefgefühlten Dank für ihr reges Interesse und vielseitige Belehrung bei der Durchführung dieser Untersuchung.

* Mitteilung aus dem Nationalen Institute für Genetik, Nr. 121 (Misima, Shizuoka-ken, Japan).

1) XXVI. Mitteil.: Bot. Mag. (Tokyo), 68: 129 (1955).

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BESCHREIBUNG DER VERSUCHE

Material und Methode

Das Pflanzenmaterial wurde in meisten Fällen in der Umgebung von Nikko gesammelt, einer der berühmtesten Stellen zur Besichtigung von herbstlich roten Blättern. Beim Einsammeln des Materials wurden wir von Herrn S. Nakamura vom dortigen Botanischen Garten vielseitig unterstützt.

Die Untersuchung der Anthocyane verlief nach dem früher beschriebenen papierchromatographischen Verfahren. Zur Extraktion des Anthocyanins benutzten wir frisch gesammelte Blätter, die ohne weiteres mit 1-2-proz. methanolischer Salzsäure ausgelaugt und innerhalb 1-2 Tage zur darauffolgenden Aufarbeitung benutzt wurden. In der Regel wurde der Farbstoff in einem 5 cm von einem Rand entfernten Linie auf einem grossen Papierstück von ungefähr 40 cm × 40 cm gestrichen und mit geeigneten Lösungsmittelgemischen entwickelt, bis eine genügende Abtrennung von Farbstoffzonen erzielt wurde. Darauf wurden die Zonen auseinandergeschnitten, und jeder der gefärbten Papierstreifen wurde, einer absteigenden Methode zufolge, vorzugsweise mit 5-proz. methanolischer Essigsäure eluiert, und das Eluat wurde dann durch Zusatz von etwas Salzsäure bis zu 1-2 Proz. angesäuert. Die erhaltene Farbstofflösung erwies sich als genügend rein. Manchmal wurde sie durch Vakuumdestillation konzentriert, und zum zweiten Chromatographieren verwendet. Ähnlich wie in voriger Mitteilung, ging die chromatographische Trennung nach der eindimensionalen, aufsteigenden Methode vor sich, wobei das TôyôFi Itrierpapier Nr. 50 oder Nr. 2 angewandt wurde. Als Lösungsmittel wurden benutzt:

Essigsäure/konz. Salzsäure/Wasser (5:1:5, v/v)	für Aglykon,
" (3:1:8, ,,)	für Glykosid,
<i>iso</i> Amylalkohol/konz. Salzsäure/Wasser (5:1:1, v/v)	für Aglykon und Glykosid,
<i>n</i> -Butanol/Essigsäure/Wasser (4:1:5, v/v)	für Glykosid,
<i>n</i> -Butanol/konz. Salzsäure/Wasser (7:2:5, v/v)	für ,, ,
Phenol/Wasser (9:1, in Gew.)	für methoxyl-haltiges Glykosid.

Selbstverständlich wurden die einzelnen Anthocyane ohne besondere Schwierigkeit identifiziert, indem wir authentische Präparate von Glykosiden bzw. deren Aglykonen auf demselben Papier chromatographierten.

Bei der Auswertung von Glykosidtypen hat sich das folgende Verfahren als gut bewährt. Zum Beispiel wurde das Farbstoffeluat mit Salzsäure bis zu ca. 20 Proz. angesäuert und einige Zeit auf dem Wasserbad bei 70° gehalten. Hierbei wurde das vorliegende Glykosid stufenweise abgebaut. Falls ein Diglykosid vorlag, verwandelte es sich nach einiger Zeit in ein Monoglykosid, das nach etwa 2 Stunden vollständig hydrolysiert wurde, was sich mittels Chromatographierens der von Zeit zu Zeit herauspipettierten Proben gut nachweisen liess. An den Monoglykosiden beobachteten wir naturgemäss kein Zwischenprodukt; sie gaben sogleich entsprechende Aglykone. Unter Benutzung dieses Verhaltens wurde es gezeigt, dass in den Herbstblättern

diglykosidische Anthocyane in meisten Fällen nicht vorliegen.

Die Hydrolysierung eines Anthocyanins erfolgte, wie früher mitgeteilt, in 20-proz. Salzsäure durch halbstündiges Erhitzen auf 90°. Nach dem Erkalten wurde das Aglykon mit etwas *iso*-Amylalkohol entzogen und auf chromatographischem Papier beschickt.

Der Nachweis der Zuckerkomponenten auf dem Chromatogramm, das aus dem Anthocyanhydrolysat nach der früher angegebenen Arbeitstechnik⁷⁾ dargestellt war, erfolgte am besten durch Bespülen mit 1-proz. äthanolischem Anilinhydrochlorid⁶⁾.

Versuchsergebnisse

Trotz der auffallenden Buntheit der herbstlichen Laubfärbungen ist das Anthocyanmuster von Blättern in der Regel nicht so verwickelt, sondern es besteht der Hauptsache nach aus einem Cyanidinmonoglykosid und zwar Chrysanthemin. Freilich gibt es aber einige Ausnahmefälle vor. Bei den Ericaceen wurde daneben auch ein Delphinidin-Derivat, und bei den Anacardiaceen und Vitaceen ein Paeonidin-Glykosid nachgewiesen. In diesem Zusammenhang ist es zu beachten, dass diese zwei Nebenfärbstoffe vielleicht in Form von Leukokörpern vorkommen, da sie gewöhnlich erst nach der Hydrolyse des Blattextraktes mit Salzsäure nachweisbar waren.

Schliesslich ist es auch bemerkenswert, dass in allen untersuchten Materialien acyliertes Anthocyanin nicht vorhanden zu sein schien.

Die gewonnenen Versuchsergebnisse sind der Übersichtlichkeit halber in nachstehender Tabelle zusammengestellt. Hier sei angegeben, dass 3-Monoglucosid, 3-Monohexosid, usw. auf chromatographischem Wege unter Anwendung der folgenden Arbeitstechnik charakterisiert wurden.

3-Monoglucosid (bzw. Chrysanthemin).....durch (a) Bestimmung von R_f -Wert, (b) papierchromatographischen Nachweis des Abbauproduktes, welches im Laufe milder Hydrolyse ausgebildet wurde, (c) Charakterisierung des Zuckers als Glucose, und (d) Vergleich mit parallellaufendem authentischem Präparat.

3-Monohexosid.....durch (a) und (b). Hierbei lag der R_f -Wert ebenfalls innerhalb des Bereiches, der für ein Monohexosid charakteristisch ist. Die Bestimmung des Zuckers war aber nicht möglich.

Monoglykosid.....Hauptsächlich durch (a); Zuckernatur war unsicher; es handelte sich um Hexose oder Pentose.

3-Hexo-pentosid.....durch (a) und (b). In der Regel lag der R_f -Wert höher als beim Monoglucosid, und bei der milder Hydrolyse liess sich das 3-Monohexosid als Zwischenprodukt nachweisen.

Glykosid.....Die Art und Zahl der Zuckerkomponenten blieben unbekannt.

Tabellarische Zusammenstellung der

Nr.	Familien	Versuchspflanzen†	Farbe d. Blätter
1	Betulaceae	<i>Carpinus laxiflora</i> Blume アカシデ	braunrot~rot
2	Fagaceae	<i>Quercus mongolica</i> Fischer var. <i>grosseserrata</i> Rehd. et Wils. ミズナラ	braun~karminrot
3		<i>Q. serrata</i> Thunb. コナラ	braunrot~rot
4	Polygonaceae	<i>Polygonum Sieboldi</i> Meisn. アキノウナギツカミ	rot
5	Cercidiphyllaceae	<i>Cercidiphyllum japonicum</i> Sieb. et Zucc. カツラ	hell rot
6	Berberidaceae	<i>Nandina domestica</i> Thunb. ナンテン	braunrot~rot
7	Saxifragaceae	<i>Hydrangea macrophylla</i> Seringe ガクアジサイ	rotpurpur
8		<i>Deutzia gracilis</i> Sieb. et Zucc. ヒメウツギ	rotbraun
9		<i>Ribes Maximowiczianum</i> Komar. ザリコミ	braunrot
10	Rosaceae	<i>Pourthiaea villosa</i> Decne. var. <i>laevis</i> Stapf カマツカ	"
11		<i>Prunus japonica</i> Thunb. ニワウメ	braunrot~rot
12		<i>P. Jamasakura</i> Sieb. ヤマザクラ	rot~dunkelrot
13		<i>P. Sargentii</i> Rehder オオヤマザクラ	rotbraun~rot
14		<i>P. yedoensis</i> Matsum. ソメイヨシノ	rot
15		<i>Rubus Koehneanus</i> Focke ミヤマニガイチゴ	orangerot~braunrot
16		<i>R. mesogaeus</i> Focke クロイチゴ	dunkel braunrot
17		<i>R. microphyllus</i> Linn. fil. ニガイチゴ	braunrot
18		<i>Sorbaria sorbifolia</i> A. Brown forma <i>incerta</i> Kitagawa エゾホザキナナカマド	dunkelrot~orangerot
19		<i>Sorbus gracilis</i> K. Koch ナンキンナナカマド	rotbraun
20		<i>Spiraea japonica</i> Linn. fil. シモツケ	bräunlich rot
21		<i>S. prunifolia</i> Sieb. et Zucc. シジミバナ	rot
22	Geraniaceae	<i>Geranium eriostemon</i> Fish. var. <i>Reinii</i> Maxim. ゲンナイフウロ	"
23		<i>G. yesoense</i> Franch. et Savat. var. <i>nipponicum</i> Nakai アカヌマフウロ	"
24	Euphorbiaceae	<i>Euphorbia pekinensis</i> Rupr. var. <i>Onoei</i> Makino タカトウダイ	braunrot
25	Anacardiaceae	<i>Rhus ambigua</i> Lavallée, ex Dippel ツタウルシ	rot
26		<i>Rh. chinensis</i> Miller ヌルデ	"
27		<i>Rh. succedanea</i> Linn. ハゼノキ	"
28		<i>Rh. trichocarpa</i> Miq. ヤマウルシ	"
29		<i>Rh. verniciflua</i> Stokes ウルシ	"

† Lateinische Pflanzennamen wurden alle von Herrn Kollegen T. Momiyama durchgesehen.

Anthocyane im japanischen Herbstlaub.

Lokalisation des Farbstoffs*	Anthocyane**	Anmerkungen. Bereits angegeben in der Literatur als:	Nr.
haupts. Palis. u. seltener ob. Epid.	Cyanidin-3-monohehexosid (10)**		1
Palis. > Schw.	" (10)		2
"	" (10)		3
ob. Epid.	" (10)		4
haupts. Palis. u. seltener Schw.	Cyanidin-diglucosid (8) + Cyanidin-3-monohehexosid (2)	Cyanidin-diglucosid (3)	5
	Cyanidin-3-monohehexosid (10)		6
	" (10)		7
ob. Epid. u. seltener unt. Epid.	Cyanidin-hexopentosid (7) + Chrysanthemin (3)	Cyanidin-pentosid (3)	8
Palis.	Chrysanthemin (10)		9
Palis., bisweilen Schw.	Cyanidin-3-monohehexosid (10)		10
Palis. > Schw., bisweilen Epid.	Chrysanthemin (10) + Cyanidinglykosid (Spur)		11
Palis., seltener Schw.	Chrysanthemin (9) + Cyanidin-hexopentosid (1)		12
Palis., seltener ob. Epid.	" (7) + " (3)	Cyanidin-3-monosid (3)	13
	Cyanidin-3-monohehexosid (10)	Cyanidin-pentosid (3)	14
	" (10)		15
Palis., seltener Schw. u. ob. Epid.	Chrysanthemin (10)		16
Palis.	Cyanidin-3-monohehexosid (10)		17
"	" (10)		18
	Chrysanthemin (10)		19
ob. Epid.	Chrysanthemin (10) + Cyanidin-monoglykosid (Spur)	Cyanidin-3-monosid (3)	20
Palis., seltener auch ob. Epid. u. Schw.	" (10) + " (Spur)		21
Palis.	Cyanidin-3-monohehexosid (10)		22
"	" (10)		23
Palis. > Schw.	Cyanidin-3-monohehexosid (10) + {Cyanidin-monoglykosid (Spur) " -glykosid (Spur)}		24
Palis.	Cyanidin-3-monohehexosid (10) + Paeonidin-monoglykosid (Spur)		25
"	Chrysanthemin (10)		26
"	Cyanidin-3-monohehexosid (10) + Paeonidin-3-monohehexosid (Spur)		27
"	" (10) + Paeonidin-glykosid (Spur)	Cyanidin-pentosid (3)	28
"	Chrysanthemin (10) + Paeonidin-glykosid (Spur)	Cyanidin-3-monosid (3)	29

* Abkürzungen: Palis. = Palisadengewebe, ob. Epid. = Oberepidermis, Schw. = Schwammgewebe.

** Ziffern in Klammern zeigen die scheinbaren Mengenverhältnisse der Anthocyane.

Nr.	Familien	Versuchspflanzen		Farbe d. Blätter
30	Celastraceae	<i>Euonymus alatus</i> Sieb.	ニシキギ	rosa-rot
31		<i>E. Sieboldianus</i> Blume	マユミ	rot
32		<i>E. oxyphyllus</i> Miq.	ツリバナ	"
33	Aceraceae	<i>Acer japonicum</i> Thunb.	ハウチワカエデ	"
34		<i>A. nikoense</i> Maxim.	メグスリノキ	"
35		<i>A. palmatum</i> Thunb. var. <i>amoenum</i> Ohwi	オオモミジ	"
36		var. <i>Matsumurae</i> Makino	ヤマモミジ	"
37		<i>A. rufinerve</i> Sieb. et Zucc.	ウリハダカエデ	"
38		<i>A. Shirasawanum</i> Koidz.	オオハウチワカエデ	"
39		<i>A. Sieboldianum</i> Miq.	コハウチワカエデ	"
40		<i>A. Tschonoskii</i> Maxim.	ミネカエデ	"
41	Rhamnaceae	<i>Rhamnus costata</i> Maxim.	クロカンバ	braunrot
42	Vitaceae	<i>Ampelopsis brevipedunculata</i> Trautv.	ノブドウ	"
43		<i>Parthenocissus tricuspidata</i> Planch.	ツタ	rot
44	Lythraceae	<i>Vitis Coignetiae</i> Pulliat	ヤマブドウ	"
45		<i>Lythrum anceps</i> Makino	ミソハギ	"
46	Oenotheraceae	<i>Epilobium pyrricholophum</i> Franch. et Savat.	アカバナ	"
47	Araliaceae	<i>Aralia elata</i> Seeman	タラノキ	dunkel violettrot
48	Cornaceae	<i>Cornus Kousa</i> Bürger	ヤマボウシ	dunkelrot
49		<i>C. controversa</i> Hemsley	ミズキ	braunrot~rot
50	Diapensiaceae	<i>Shortia soldanelloides</i> Makino var. <i>ilicifolia</i> Makino	ヒメイワカガミ	bräunlich rot
51	Clethraceae	<i>Clethra barbinervis</i> Sieb. et Zucc.	リョウブ	orangerot~braunrot
52	Ericaceae	<i>Enkianthus campanulatus</i> Nichols.	サラサドウダン	braunrot~rot
53		<i>E. perulatus</i> Schneider	ドウダンツツジ	dunkelrot
54		<i>E. rubicundus</i> Matsumura et Nakai	ベニサラサドウダン	rot
55		<i>Rhododendron dauricum</i> Linn.	エゾムラサキツツジ	bräunlich rot
56		<i>Rh. japonicum</i> Suringar	レンゲツツジ	dunkelrot
57		<i>Rh. Kaempferi</i> Planch.	ヤマツツジ	braunrot~rot
58		<i>Rh. Keiskei</i> Miq.	ヒカゲツツジ	braunrot

Lokalisation des Farbstoffs	Anthocyane	Anmerkungen. Bereits angegeben in der Literatur als:	Nr.
ob. Epid. u. seltener unt. Epid.	Chrysanthemin (10)	Cyanidin-3-monosid (3)	30
Palis. > Schw.	" (10)		31
ob. Epid. u. seltener Mesophyll	" (10)	Cyanidin-monosid (3)	32
	" (10)		33
haupts. Palis. u. seltener Schw.	" (10)		34
Palis. > Schw., bisweilen Epid.	" (10)		35
Palis. > Schw.	" (10)	Chrysanthemin (1)	36
Palis.	" (10)	Cyanidin-3-monosid (3)	37
haupts. Palis. u. seltener ob. Epid.	" (10)	Chrysanthemin (1)	38
Palis. > Schw., seltener Epid.	" (10)	"	39
"	" (10)	Cyanidin-pentosid (3)	40
haupts. Palis., seltener Epid. u. Schw.	" (10)		41
	Chrysanthemin (5) + Paeonin (3) + Cyanin (2)		42
haupts. Palis., seltener Epid.	Cyanidin-3-monohehexosid (10)		43
"	Chrysanthemin (6) + Cyanidin-dihexosid (4)	Cyanidin-3-monosid (3)	44
Palis.	Cyanidin-3-monohehexosid (10)		45
	" (10)		46
	Cyanidin-glycosid (7) + anderes Cyanidin-glykosid (1) + Cyanidin-3-monohehexosid (2)		47
Palis.	Cyanidin-3-monohehexosid (10)	Cyanidin-3-monosid (3)	48
"	" (10)		49
Palis. > Schw.	" (10)		50
Palis.	Cyanidin-3-hexo(?)pentosid (5) + Cyanidin-glykosid (5)		51
haupts. Palis. u. seltener Schw.	Cyanidin-3-monohehexosid (7) + Cyanidin-monoglykosid (3) + Leuco-delphinidin (<i>Spur</i>)		52
Palis., bisweilen auch Epid.	Cyanidin-3-monohehexosid (9) + Cyanidin-monoglykosid (1)	Cyanidin-monosid (3)	53
Palis. u. seltener Schw.	Cyanidin-3-monohehexosid (10) + Cyanidin-monoglykosid (<i>Spur</i>)		54
	Cyanidin-monoglykosid (6) + Cyanidin-3-monohehexosid (4)		55
Palis. u. seltener Schw.	Cyanidin-3-monohehexosid (5) + Cyanidin-monoglykosid (5) + Leuco-delphinidin (<i>Spur</i>)		56
Palis.	Chrysanthemin (10) + Delphinidin-3-monohehexosid u. Leuco-delphinidin (<i>Spur</i>)		57
Palis. > Schw.	Cyanidin-3-monohehexosid (4) + Cyanidin-monoglykosid (6) + Leuco-delphinidin (<i>Spur</i>)		58

Nr.	Familien	Versuchspflanzen	Farbe d. Blätter
59		<i>Rhododendron nikoense</i> Nakai アカヤシオ	rot
60		<i>Rh. quinquefolium</i> Bisset et Moore シロヤシオ	"
61		<i>Rh. Schlippenbachii</i> Maxim. グロフネツツジ	dunkel braunrot
62		<i>Rh. semibarbatum</i> Maxim. バイカツツジ	braunrot~rot
63		<i>Rh. Tschonoskii</i> Maxim. コメツツジ	dunkelrot~rot
64		<i>Rh. Wadanum</i> Makino トウゴクミツバツツジ	rotbraun~rot
65		<i>Tripetaleia paniculata</i> Sieb. et Zucc. ホツツジ	dunkel violettrot
66	Ebenaceae	<i>Diospyros Kaki</i> Thunb. カキ	rot
67	Oleaceae	<i>Ligustrum obtusifolium</i> Sieb. et Zucc. イボタ	dunkel violettrot
68		<i>Osmanthus ilicifolius</i> Standish ヒイラギ	braunrot
69		<i>Syringa Palibiniana</i> Nakai チョウセンハシドイ	scharlachrot
70	Verbenaceae	<i>Callicarpa japonica</i> Thunb. ムラサキシキブ	orangerot
71	Caprifoliaceae	<i>Viburnum dilatatum</i> Thunb. ガマズミ	bräunlich rot
72		<i>V. furcatum</i> Blume オオカメノキ	"
73		<i>V. urceolatum</i> Sieb. et Zucc. var. <i>procumbens</i> Nakai ミヤマシグレ	"
74		<i>V. Wrightii</i> Miq ミヤマガマズミ	"

Zusammenfassung

1. An verschiedenen rot bis rotbraunen Herbstblättern wurden die Anthocyane mit Hilfe von Papierchromatographie untersucht.

2. Zu dieser Untersuchung wurden 74 Pflanzen aus 25 Familien herangezogen, welche die schönste Herbstströtung von Blättern im Gebirgsgegenden Mitteljapans aufweisen.

3. Trotz der Unterschiede in der Pigmentierung war die Anthocyanzusammensetzung einfach; der Farbstoff der herbstlich roten Blätter bestand hauptsächlich aus einem Cyanidin-monoglykosid bzw. Chrysanthemin.

Lokalisation des Farbstoffs	Anthocyane	Anmerkungen. Bereits angegeben in der Literatur als:	Nr.
Palis.	Cyanidin-monoglykosid (6)+Cyanidin-3-mono-hexosid (4)		59
"	Chrysanthemin (10)		60
Palis. u. seltener Schw.	Cyanidin-monoglykosid (8)+Cyanidin-3-mono-hexosid (2)+Leuco-delphinidin (<i>Spur</i>)	Cyanidin-monosid (3)	61
Palis.	Chrysanthemin (5)+Delphinidin-3-monoglykosid (5)		62
"	Cyanidin-3-mono-hexosid (5)+Cyanidin-glykosid (5)+Leuco-delphinidin (<i>Spur</i>)		63
"	Cyanidin-3-mono-hexosid (10)+Leuco-delphinidin (<i>Spur</i>)		64
"	" (10)		65
Palis. u. seltener auch Schw.	" (10)		66
haupts. Palis., seltener ob. Epid.	Chrysanthemin (4)+3 Arten des Cyanidin-glykosids (3:2:1)	Cyanidin-3-pentosid (3)	67
	Cyanidin-3-mono-hexosid (10)		68
	Chrysanthemin (10)+Cyanidin-glykosid (<i>Spur</i>)		69
ob. Epid.	Cyanidin-3.5-di-hexosid (6)+Cyanidin-3-mono-hexosid (2)+Cyanidin-glykosid (2)	Cyanidin-3.5-dimonosid (3)	70
Palis.	Cyanidin-3-mono-hexosid (10)	Cyanidin-3-monosid (3)	71
Palis. (ob. Schicht)	Chrysanthemin (10)		72
haupts. Palis. u. seltener Schw.	" (10)		73
Palis.	Cyanidin-3-mono-hexosid (10)		74

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Flowering Response to Various Combination of Light and Dark Periods in *Silene Armeria*

by Atsushi TAKIMOTO*

滝本 敦：種々の明暗週期下に於けるムシトリナデシコの花芽形成

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Many important informations on photoperiodism have been obtained from studies on the developmental behavior of plants receiving various cycles of light and darkness of other than the normal 24-hours' duration^{1, 3, 5, 6)}. From extensive investigations of Biloxi soy bean and *Xanthium pennsylvanicum*, Hamner came to the conclusion that reactions taking place both in light and darkness may be involved in photoperiodic induction⁵⁾. Also with long day plants such experiments have been carried out by several workers^{1, 3, 5)} but in comparison with the short day plants many points remained to be more thoroughly examined.

In the present investigation floral initiation in *Silene Armeria* under various combinations of light and dark periods other than the normal 24 hours' cycle was examined by the use of artificial light.

Material and Methods

Silene Armeria, sown in September, continues a strictly vegetative growth under short photoperiod until June of the next year. But when it is exposed to continuous illumination supplemented with artificial light at night, floral initiation occurs within 5-10 days.

From early September to November, the seeds were sown in several intervals in the field. Forty-nine plants ——— in some cases thirty-five ——— whose leaves had reached the length of 1-1.5 cm were selected for uniformity and transplanted in 30 cm×20 cm×10 cm wooden boxes. After transplantation they were grown in the greenhouse under short day condition, being exposed to natural day length in winter, but placed in a light-proof compartment from 5 p.m. to 9 a.m. in early spring. 2-3 weeks after the transplantation, they were employed in the experiments. At that time, they had developed several leaf pairs of 2-2.5 cm length, and had a high sensitivity to photoperiodic induction. The sensitivity varied considerably with the season. In early spring they showed the highest and in cold winter the lowest

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sensitivity. In every experiment a control lot exposed to a cycle consisting of 16 hours of light and 8 hours of darkness was provided to serve as standard for the photoperiodic sensitivity. The experiments reported here were carried out in late winter of 1953 and in early spring of 1954.

Alternation of light and darkness was secured with the help of an automatic clock-work in eight light-proof cabinets measuring 70 cm×70 cm×45 cm, placed in a cellar room where the temperature was maintained at 20°~21° C. Each cabinet was furnished with electric lamps and ventilated thoroughly to avoid high temperature, but rise to 22°~23° C, during the illumination period could not be avoided. Lamps served as source of illumination, namely two Mazda day light fluorescent lamps of 20 watt, one Mazda pink fluorescent lamp of 20 watt and one Mazda incandescent filament lamp of 20 watt. Light intensity at the leaf surface was about 210 foot candles. Preliminary experiments indicated that various sources of light had a remarkably different effect on photoperiodic induction in *Silene Armeria*. Further examination on this point was not carried out, but the above mentioned combination was found to be the most suitable so far as investigated.

Plants to be treated were placed at 5 p.m. in the cabinet, in which they were held in darkness until 9 a.m. of the next morning. Therefore, they received a dark period of at least 16 hours and thereupon they were subjected to the first light period of any particular treatment. They were subsequently exposed to various planned cycles of light and darkness for ten days. At the close of the treatments the plants were again held at least 16 hours in darkness, and then returned to the greenhouse, where they were allowed to develop under short day condition.

During the experiments some plants were discarded on account of fungous injury and excluded from further observation. About 3-4 weeks after the close of treatments, the plants were carefully examined for the initiation of floral primordia at the terminal buds, and developmental stages of floral primordia were recorded. At the same time the stem length was also measured.

The flower primordia were classified in seven groups, from 1/2 to 6, according to their developmental stage. The vegetative growing point was designated as O: it is dome shaped and successively gives rise to decussate leaves. In this way each of the seven stages of Fig. 1 had a numerical value and it was easy to calculate the average stage for the individual plants. The first reliable sign of floral initiation is the appearance of a tubercular process on one side of the growing point (Stage 1/2). Such a tubercular process soon appears on the opposite side too, forming three growing points in one plane (Stage 1). The growing point in the middle develops into a terminal flower. Each lateral process develops again two tubercular processes in the plane perpendicular to that of the preceding branching, and this mode of branching is repeated to form a dichasium. The number of floral primordia thus formed in a terminal inflorescence is mainly dependent on the size of the growing point, which is determined by the vigor of the plant. But the developmental

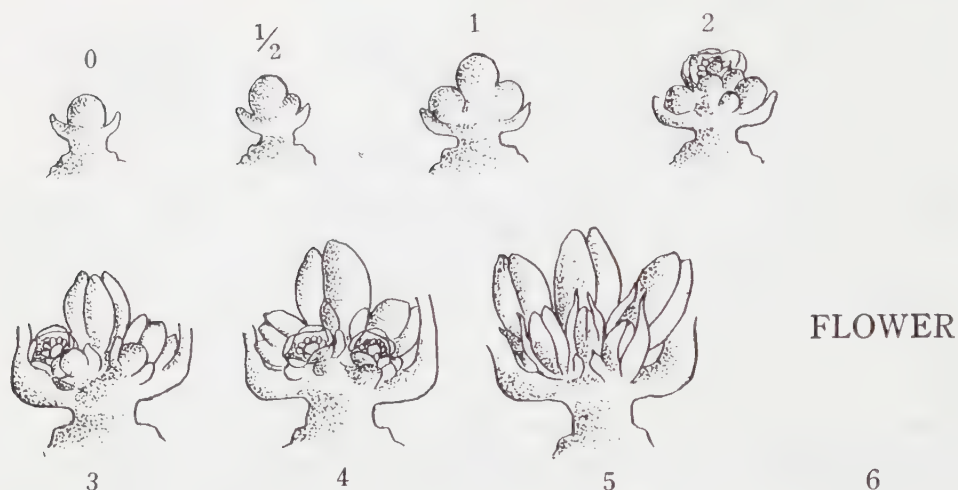


Fig. 1. Developmental stages of floral primordia in *Silene Armeria*.

rates of the individual floral primordia are mainly concerned with the intensity of floral induction. Therefore, when the later stages, 3-5 in Fig. 1 were determined, emphasis was laid on the development of floral organs in the early formed floral primordia of the inflorescence. In all experimental and control lots, the average numerical value of the developmental stages of the individual plants was used to indicate the intensity of floral induction.

Experimental results

The plants were subjected to the following photoperiods for ten days.

- 1) 4 hours light and 4, 8, 10, 12 and 14 hours darkness.
- 2) 8 hours light and 8, 10, 12, 14 and 16 hours darkness.
- 3) 12 hours light and 6, 8, 10, 12, 14, 16 and 24 hours darkness.
- 4) 14 hours light and 8, 12, 16 and 24 hours darkness.
- 5) 16 hours light and 8, 12, 16, 20, 24 and 36 hours darkness.
- 6) 24 hours light and 16, 24 and 36 hours darkness.
- 7) 10 days continuous illumination.
- 8) 10 days continuous darkness.

Because of the limited number of cabinets used, it was impossible to get all desired cycles of light and darkness at the same time. In each experimental series a control lot was subjected to a cycle consisting of 16 hours of light and 8 hours of darkness, to serve as standard for photoperiodic sensitivity.

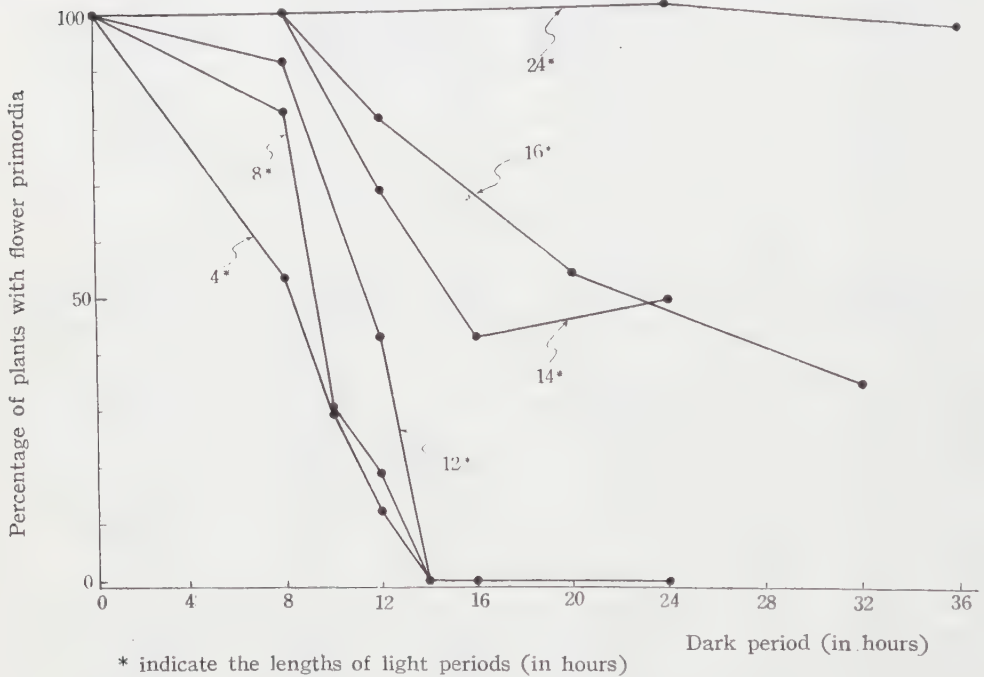
The results are shown in Table 1. For brevity, in this table, the photoperiodic cycles are represented by abbreviations. For example, (4^h1+4^hd) indicates a cycle consisting of 4 hours of light and 4 hours of darkness. 10^d1 and 10^dd indicate continuous illumination for 10 days and continuous darkness for 10 days, respectively.

Table 1. Flower initiation of *Silene Armeria* exposed to various cycles of light and darkness for ten days.

Light-darkness cycles	No. of plants used	% of plants with flower primordia	Average stage of flower primordia	Average length of stems in mm.	Dates of sowing, trans-plantation, treatment, and dissection.
4h 1+4h d	47	100	4.2	42.7	7/X,
4h 1+6h d	49	93.9	3.3	39.5	29/XII,
4h 1+8h d	46	41.3	2.5	28.2	18/I~28/I
4h 1+12h d	49	26	1.8	24.0	26/II
16h 1+8h d	47	100	3.1	38.8	
4h 1+8h d	32	53.1	2.7	59.1	5/XI,
4h 1+10h d	37	29.7	2.5	60.5	24/II,
4h 1+12h d	42	11.9	1.6	38.5	11/III~21/III
4h 1+14h d	45	0	0	44.1	5/IV
16h 1+8h d	35	100	3.9	66.4	
8h 1+8h d	48	50	2.3	18.4	5/XI,
8h 1+10h d	40	65.0	2.4	20.5	17/XII,
8h 1+12h d	48	27.1	2.3	12.8	8/I~18/I
8h 1+14h d	48	0	0	9.7	17/II
8h 1+16h d	33	0	0	9.5	
16h 1+8h d	47	93.6	3.1	29.8	
8h 1+8h d	34	82.4	2.9	32.8	5/XI,
8h 1+10h d	30	30.0	2.2	33.1	4/III,
8h 1+12h d	27	18.5	1.4	25.3	21/III~31/III
8h 1+14h d	21	0	0	28.7	19/IV
8h 1+16h d	33	0	0	32.6	
16h 1+8h d	34	100	4.2	71.5	
12h 1+6h d	33	87.9	4.1	45.6	5/XI,
12h 1+8h d	34	85.3	3.9	46.9	14/I,
12h 1+12h d	34	29.4	2.5	34.1	4/II~14/II
12h 1+14h d	33	0	0	26.2	5/III
16h 1+8h d	34	91.2	3.8	65.9	
12h 1+8h d	35	91.4	4.1	56.2	5/XI,
12h 1+12h d	33	42.4	2.4	38.0	4/II,
12h 1+14h d	33	0	0	23.5	28/II~10/III
12h 1+16h d	33	0	0	22.4	23/III
12h 1+24h d	20	0	0	15.7	
16h 1+8h d	34	100	4.3	61.5	
14h 1+8h d	35	100	3.4	68.8	5/XI,
14h 1+12h d	32	71.9	2.6	69.9	24/II,
14h 1+16h d	40	42.5	2.5	53.5	11/III~21/III
14h 1+24h d	35	48.6	1.8	44.2	5/IV
16h 1+8h d	35	100	3.9	66.4	
16h 1+8h d	47	100	3.1	38.8	7/X,
16h 1+12h d	48	83.3	2.7	30.8	29/XII,
16h 1+16h d	47	70.9	3.1	40.8	18/I~28/I
16h 1+24h d	48	39.6	2.6	27.5	26/II
16h 1+8h d	36	100	3.9	32.3	5/XI,
16h 1+12h d	32	81.2	3.7	31.1	24/I,
16h 1+20h d	41	53.7	2.5	30.8	17/II~27/II
16h 1+32h d	39	33.3	2.1	26.9	16/III
24h 1+16h d	42	100	3.8	42.5	5/XI
24h 1+24h d	38	100	2.7	36.4	24/I,
24h 1+36h d	34	94.1	2.2	34.4	17/II~27/II
16h 1+8h d	36	100	3.9	32.3	16/III
10d 1	49	100	4.5	55.0	7/X,
10d d	18	0	0	30.4	1/XII,
16h 1+8h d	40	37.5	2.0	22.2	23/XII~2/I
					31/I
10d 1	20	70.0	3.3	31.5	7/X,
10d d	18	0	0	29.9	12/XI,
16h 1+8h d	20	40.0	3.0	21.4	2/XII~12/XII
					11/I

Plants exposed to continuous illumination for ten days initiated floral primordia most easily, and those exposed to continuous darkness for ten days initiated no floral primordia. In each experiment performed between February 17th and March 31st, the control lots exposed to the cycle consisting of 16 hours of light and 8 hours of darkness showed similar sensitivity to photoperiodic induction. Data obtained from these experiments are summarized in Fig. 2. Exposed to cycles of constant

Fig. 2. Relations between percentages of plants with flower primordia and lengths of dark periods, under the cycles with light periods of 4, 8, 12, 14, 16 and 24 hours.



dark periods and various light periods with the increase of the latter more plants initiated floral primordia whose developmental stages were in addition more advanced. This was also the case with decreasing dark periods when exposed to cycles of constant light periods followed by various dark periods. The most significant fact is that if the light periods are shorter than 12 hours floral initiation occurs only when the related dark periods are less than 12-14 hours, but if the light periods are extended to 14 hours or more, floral initiation occurs even when the following dark periods are extended to 24 hours or more. Under cycles with a light period of 24 hours almost all plants — 33 out of 34 — initiated floral primordia even if the following dark periods were extended to as many as 36 hours.

Stem length varies considerably with the experimental seasons, probably being affected by the temperatures in the greenhouse. But in each experimental series, generally, the longer was the stem, the higher was numerical value of the stage of the floral primordia.

Discussion

In long day plants it is uncertain whether the light period has a promoting effect or the dark period an inhibitory effect upon floral initiation. Hitherto many ideas have been proposed to interpret their photoperiodic behavior^{2, 4, 5, 7, 8}.

Floral initiation in *Silene Armeria* occurs most easily under continuous illumination, and it becomes progressively difficult with the increase of an included dark period. No floral primordia are initiated when the plant is exposed to continuous darkness for ten days. It seems that reactions which induce floral initiation proceed only during the light period. The present data shows that under cycles comprising a light period of less than 12 hours floral initiation does not occur if the following dark period runs 14 hours or longer, but under cycles with light periods of 14 hours or more, floral initiation occurs even if the following dark periods run 24 hours or more. It seems that if the light period lasts less than 12 hours, the following dark period is the determining factor which completely inhibits floral initiation when it is continued for 14 hours or more, but the light period becomes a determining factor if it runs 14 hours or more, and floral initiation occurs in spite of the following long dark period. Presumably, a dark period tends to destroy the changes which have taken place in the preceding light period, but if the light period is extended beyond a certain limit (12-14 hours), further changes had taken place which make the plant insensitive to the following dark period.

From the present data obtained in *Silene Armeria* the photoperiodic behavior of many other long day plants can be interpreted. For instance, Allard and Garner examined floral initiation of *Rudbeckia bicolor* under equal alternations of light and darkness, in eight cycles ranging from 10 to 36 hours⁹. In this case, only the plants receiving 12 hour alternations of light and darkness failed to flower, but other plants receiving 5-, 8-, 13-, 14-, 15-, 16- and 18 hours' alternations flowered. This may be interpreted as follows: in *Rudbeckia bicolor*, under cycles comprising shorter periods than 12 hours, the dark period is the determining factor of floral initiation and when it is maintained for 12 hours flower initiation does not occur, but under cycles with periods in excess of 12 hours, namely at 13-, 14-, 15-, 16- and 18-hours alternation, the light period is the determining factor and floral initiation occurs in spite of the following long dark period of more than 12 hours.

It is interesting to compare the photoperiodic behavior of *Silene Armeria* with that of short day plants. Short day plants, for example Biloxi soy bean, initiate floral primordia only when the light period is less than 20 hours and the following dark period is longer than 10½ hours⁶. *Silene Armeria* is inhibited in the initiation of floral primordia only when the light period is less than 12 hours and the dark period is longer than 14 hours. Namely, cycles consisting of a light period of less and dark period of more than a certain number of hours seem to promote floral initiation in short day plants, but inhibit floral initiation in long day plants.

Photoperiodic conditions required for floral initiation of long day plants seem to be of an opposite kind to those of short day plants. It is conceivable that the opposite effect is based on a quantitative rather than a qualitative type of difference, and the action of photoperiods consists in adjusting the balance of some processes participating in floral initiation.

Summary

Floral initiation in *Silene Armeria*, a long day plant, was examined under various light-darkness cycles other than the normal 24 hours' cycle. Artificial light was used. The results are summarized as follows:

1) Under the cycles of constant dark periods alternating with various light periods, more plants initiated floral primordia, and their developmental stages were also more advanced, with increasing length of the light period. This was also the case when the dark periods were decreasing under cycles of constant light periods followed by various dark periods.

2) If the light period was less than 12 hours, floral initiation did not occur when the following dark period continued for 14 hours or more, but if the light period was 14 hours or more, floral initiation occurred even when the following dark periods were extended up to 24 hours or more.

Grateful acknowledgment is given to Professor S. Imamura for his suggestions and criticisms.

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植物組織における TTC 還元について

補 足 的 報 告*

佐 藤 七 郎**

Sitiro SATO: A Supplemental Report on the Reduction of TTC
by Plant Embryo Slices.

1955 年 6 月 30 日受付

著者はさきに⁷⁾、植物胚の切片による TTC 還元反応にたいするコハク酸塩、モノヨードサク酸、マロン酸塩、サク酸塩、青酸塩および熱、Ca, Pb イオンの作用を検討し、ある特定の条件のもとでは、TTC (2, 3, 5-triphenyl tetrazolium chloride) 還元反応によつて、コハク酸脱水素酵素活性の組織内分布をを検出することが可能であることを結論した。

しかしながら、組織化学の手法は条件の複雑さのために誤つた結論に陥る危険が大きいので、条件を異にした実験を重ねて検討しなければならない。文献にも、リンゴ酸、乳酸、グリセロリン酸¹⁾、アルコール⁸⁾などを基質とする脱水素酵素の検出は可能であるが、コハク酸脱水素酵素には適用でさない^{2,3)}というものもすくなくない。著者は前報の実験を補つて、さらにいくつかの反応条件の検討をくわえ、さきの結論を支持する結果をえたので補足として報告する。

材 料 と 方 法

材料にはマスターピースとよばれるツルナシインゲン *Phaseolus vulgaris* の一品種をえらんだ。乾燥したタネを水に浸して約 20 時間後幼茎・幼根をカミソリで 4~5 枚にうすくたてぎりにする。この切片を 4~5 枚ずつ、反応液 5 cc とともに 10 cc 秤量ビンにいれ、38°C のフラン器中で加温する。フラン器にいたれた時刻を起点として、時間をおつて発色を記録する。反応の程度の表示は前報に準ずる。反応液は、あらかじめ原液 (10⁻¹

M リン酸緩衝液 90 容+10⁻¹ % TTC 水溶液 10 容) をつくり、これの 4 cc に基質と水を加えて 5 cc としたものを対照実験に用い、処理実験には水のかわりに薬物を加えたものを用いた。

実 験

1. アヒ酸塩

Table 1. Effect of arsenite on the reduction of TTC in the presence or absence of succinate.

Succinate Sodium arsenite	10 ⁻² M		None	
	10 ⁻³ M	None	10 ⁻³ M	None
Time(min.)				
15	—	—	—	—
30	—	—	—	—
45	—	—	—	—
65	—	+	—	±
75	—	+	—	±
100	—	++	—	+
120	—	+++	—	++

Table 2. Effect of 10⁻³ and 10⁻⁴M arsenite on the reduction of TTC in the presence of succinate.

Concn. of arsenite	10 ⁻³ M	10 ⁻⁴ M	None
Time (min.)			
13	—	—	—
23	—	?	±
40	—	+	+
60	—	+	++
85	—	++	+++
100	—	++	+++

* 日本植物学会第 18 回大会 (1953) において発表したものの一部。

** 東京大学理学部植物学教室細胞学研究室 業績第 号

コハク酸脱水素酵素を阻害するといわれている⁹⁾アヒ酸塩の作用を検討した。Tab. 1 は、基質としてコハク酸ソーダを加えたばあいも加えないばあいも、最終濃度 $10^{-3}M$ のアヒ酸塩は完全に発色を阻止することを示す。

$10^{-4}M$ になると阻害はごくわずかになつてしまう (Tab. 2)。

2. ピロリン酸塩

ピロリン酸もコハク酸脱水素酵素の阻害剤といわれている^{9, 10)}。しかし、反応液に $10^{-2}M$ のピロリン酸ソーダをふくませたのでは、Roberts, L. W.⁹⁾と同様にならえいきようがみられない。同じ胚からとつた切片について同時におこなつた対照実験と同じ速さで反応が進行する。これはピロリン酸塩にたいする細胞の透過性がひくいためとおもわれるので、前処理をどこして透入をたすけた。前処理としては、切片を反応液にいれるまえに、 $10^{-2}M$ ピロリン酸ソーダ ($10^{-1}M$ リン酸緩衝液 pH 6, 8 にとかす) に浸し、 $38^{\circ}C$ のフラン器で 60 分間加温。60 分後、ただちに $10^{-2}M$ ピロリン酸塩をふくむ反応液にうつして、 $38^{\circ}C$ で反応の進行を対照実験と比較する。対照実験は、おなじ胚からとつた切片を緩衝液に浸して同じ温度にたもつたものを、60 分後に、阻害剤をふくまない反応液にうつして、 $38^{\circ}C$ で反応をおこさせる。その結果は Tab. 3 である。

Table 3. Slight inhibition of TTC reduction by pretreatment with buffered $10^{-2}M$ sodium pyrophosphate solution at $38^{\circ}C$ for 60 min.

Time (min.)	Pretreated with sod. pyrophosph.	Control (Pretreated with buffer soln.)
10	—	—
25	—	—
40	?	±
50	±	+
75	±	+

対照実験じたいも反応がよわいが、前処理した方はさらによわく、やや阻害があつたことがうかがえる。しかし阻害作用はまだ明確でない。

つぎに前処理の時間をのばして 11 時間とした

ばあい、阻害はきわめて明瞭である (Tab. 4)。

Table 4. Complete inhibition of TTC reduction by prolonged (11 hrs.) pretreatment with buffered $10^{-2}M$ sodium pyrophosphate solution.

Time (min.)	Treated	Control
15	—	—
35	—	+
60	—	+

前処理をさらに徹底させるために、切片を $10^{-1}M$ ピロリン酸ソーダ水溶液に沈めて、これを水流ポンプで減圧し、細胞間隙の空気をおいだして溶液をしみとおらせる。対照は蒸溜水中で同一の操

Table 5. Decrease of TTC reducibility after the evacuation of slices for 30 min. in buffered $10^{-2}M$ pyrophosphate solution.

Time (min.)	Evacuated in	
	pyrophosph. soln.	dist. water
16	—	—
38	±	±
53	±	+
93	±	+
122	±	+++

Table 6. Complete inhibition of TTC reduction after the prolonged evacuation (60 min.) in buffered pyrophosphate solution.

Time (min.)	Evacuated in	
	pyrophosph. soln.	dist. water
18	—	—
24	—	—
36	—	—
46	—	—
60	—	±
67	—	+

作をおこなう。排気 30 分ののち、前者をピロリン酸塩をふくむ反応液に、後者をそれをふくまぬ

反応液にせずめて 38° C で反応させる。結果 (Tab. 5) は阻害作用がみとめられる。排気中の時間を延長して 60 分としたときは、阻害作用はほぼ完全に達した、ただしこのばあいには、たんなる水の浸透による害もかなりみられた (Tab. 6)。

よわいもので、両緩衝液を等量ずつまけたものでは、ほとんど阻害はみとめられなくなる。

4. pH のえいきよう

pH 3.8~8.0 の 10⁻¹M McIlvaine 緩衝液を用いて pH のえいきようをしらべた。結果を Tab.

Table 7. Comparison of TTC reduction in Sprensen buffer and in McIlvaine buffer.

Buffer pH	Sorensen		McIlvaine	
	5.6	6.8	5.6	6.8
Time (min.)				
10	—	?	—	—
20	—	++	—	+
35	+	+++	—	++
55	++	+++	+	++
75	+++	++++	+	+++
120	++++	+++++	++	+++

3. クエン酸塩

リン酸緩衝液のかわりにクエン酸をふくむ McIlvaine 緩衝液をつかい、pH 5.6 と 6.8 において、クエン酸基のえいきようを比較した (Tab. 7)。

pH 値が高いほうが反応がはやいことは後の実験と同じだが、いずれの pH 値においてもクエン酸基によれい阻害作用がある。しかしこの阻害は

8, 9 に表示する。

pH 5.0 以下では反応がいちじるしく阻害され、pH 値が高くなるほど反応がつよい。

5. 2,4-D

2,4-D は炭水化物の代謝を促進するといわれている。結果はわずかな促進を示した (Tab. 10)。

6. 切片の厚さ

Table 8. Effect of pH on the reduction of TTC.

pH	3.8	5.0	6.8	8.0
Time (min.)				
30	—	—	—	—
45	—	—	+	+
82	—	?	++	+++
100	—	+	+++	++++
135	—	+	++++	++++

Table 9. Effect of pH on the reduction of TTC.

pH	5.0	5.8	6.8	8.0
Time (min.)				
20	—	—	+	+
40	—	?	+++	++++
60	?	+	+++	+++++
85	?	++	++++	+++++

Table 10. Effect of 2,4-D on the reduction of TTC.

2,4-D	0.002%	None
Time (min.)		
10	—	—
23	±	—
38	+	±
58		±
62	+	+

組織をすりつぶすと、反応はいちじるしくよめられる。また、切片がうすすぎると、反応がすまないと いわれている⁵⁾ し経験的にもしられたので、シリンダーマイクロームを用いて厚さ 108 μ , 144 μ , 216 μ の三通りの切片をつくり、標準反応液で反応させて比較した。

Table 11. Comparison of TTC reduction by sections in various thickness.

Thickness (μ)	108	144	216
Time (min.)			
14	—	?	+
27	—	?	+
45	—	±	++
60	—	±	++
100	—	±	+++

結果 (Tab. 11) は 140 μ ぐらいが限度で、そ

れ以下になると反応がいちじるしく減退する。

7: ふたたび KCN の作用について

前報で、 $10^{-3}M$ ていどの KCN が阻害作用をもっていることを明かにした。そして TSOU を引用し、KCN が直接にコハク酸脱水素酸素を阻害する可能性を考えた。しかし Tsou¹⁰⁾ は腎、心筋の均質液のメチレン青脱色にたいして比較的高濃度 ($5 \times 10^{-3}M$) で非可逆的な不活性化作用があるという。著者の実験条件における KCN の害作用はきわめて明瞭であるので、この害作用の特性をくわしくすることが重要であると考え、つぎの実験をおこなつた。

KCN を 10^{-1} , 10^{-2} , 10^{-3} , $10^{-4}M$ の濃度に、 $10^{-1}M$ リン酸緩衝液 (pH 6.8) にとかした前処理液を 2 組つくる。対照実験用には KCN をふくまぬ緩衝液を用う。これらの各 5 cc に切片をひたし、30 分間後、いつせいに 5 分間緩衝液で洗う。ついで KCN をふくむ緩衝液で前処理した 2 組のうち、1 組をそれぞれ 10^{-1} , 10^{-2} , 10^{-3} , $10^{-4}M$ KCN をふくむ反応液 (原液 4.0 cc + KCN 0.5 cc + 水 0.5 cc; 基質をふくまず) にうつす。他の 1 組はいずれも KCN をふくまぬ反応液 (原液 4.0 cc + 水 1.0 cc) にうつし、それぞれにたいする対照実験とする。はじめ KCN をふくまぬ緩衝液で前処理した切片は、そのままやはり KCN をふくまぬ反応液にうつして、ぜんたいを通じた無処理の対照とする。

結果 (Tab. 12) は基質をふくまぬために、ぜん

Table 12. Effect of KCN on the reduction of TTC.

Pretreatment (30 min.)	Concn. (M) of KCN in buffer						
	10 ⁻¹		10 ⁻²	10 ⁻³	10 ⁻⁴		0
Incubation	Concn. (M) of KCN in react. mix.						
	10 ⁻¹	0	10 ⁻²	0	10 ⁻³	0	0
Time (min.)							
20	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—
45	—	—	—	—	—	+	+
61	—	—	—	?	—	++	++
71	—	+	—	+	—	+++	+++
76	—	+	—	++	—	+++	+++
101	—	++	—	+++	—	+++	+++
144	—	++++	—	++++	—	++++	++++

たいとして反応のあらわれ方がおそいが、前処理の KCN の濃度が大きいほど阻害が大きい。前処理と反応液に KCN を与えられたばあいには、 10^{-4} M という低濃度でも反応が完全にとまっている。

KCN の前処理だけをうけて、反応中に KCN を与えられないばあいには、水洗いによつて阻害が回復する。 10^{-4} M では完全に回復して、前処理をうけないばあいと区別できない。それ以上の濃度でもいくぶん回復し、長時間後には青色する。すなわち、KCN の作用は可逆的である。 10^{-2} M 以上の KCN で前処理したばあいには、もはやあるていど以上には回復しない。すなわち回復に限度がみられる。

これらの阻害様式は、濃度の点においても可逆性の点においても、Tsou の結果と一致しない。よつて、この実験における KCN の阻害作用は、

べつの機作をもつて説明されなければならない。それは TTC の還元に関与する酵素だけでなく、間接になんらかの関係をもつ諸酵素、および細胞原形質の膠質化学的条件、細胞の透過性などにたいする作用をも考慮にいれて解析さるべきであろう。

ま と め

実験をおこなつたかぎりでは、コハク酸脱水素の特性が結果にあらわれた。したがつて、前報の結論とおなじように、TTC 反応によつて植物のコハク酸脱水素酵素活性の組織化学的検出が可能であること、および発芽初期の幼胚では、この酵素活性は前形成層の細胞につよいことが、ふたたび確認された。KCN の作用については、さらにくわしい定量的実験が必要とおもわれる。

Summary

The effects of arsenite, pyrophosphate, citrate, pH, 2, 4-D, thickness of slices and cyanide on the TTC reduction in the slices of radicle and hypocotyl of seedlings of *Phaseolus vulgaris* were examined. Among these reagents and reaction conditions, arsenite, pyrophosphate, cyanide and low pH inhibited the reduction of TTC, while 2, 4-D accelerated it slightly. Citrate had no apparent effect on it. When the slices were thinner than about 150μ , no reduction appeared in them even under the best conditions. The inhibition by cyanide and its partial reversibility were confirmed. It was concluded that the TTC method for the histochemical demonstration of succinic dehydrogenase in plant tissues is reliable, and that the enzyme is distributed in the cells of procambial tissue.

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北海道アポイ山の蛇紋岩地苔類*

服 部 新 佐^{*†}

Sinske HATTORI: Hepaticae Occurring on Serpentine on Mt. Apoi (Hokkaido)

1955 年 8 月 26 日受付

昨夏清水大典氏^{***}が採集した北海道の苔類標本約 1000 点の研究が終つたので、その中より日高支庁のアポイ山 (810 m) 蛇紋岩地苔類に関するデータを抜粋して簡単に報告する。筆者は野口彰博士等と協力して日本産蘚苔類とその著生基物 (乃至は広く蘚苔類の分布と地質) との関係に就き数年来研究中であるが、我国蛇紋岩の蘚苔類フロラに関する研究発表は未だ見られず、且つ海外に於ける研究も余り見る可きものが無いようである。

蘚苔類は一般高等植物よりも遙かに基岩に対して鋭敏であることは言う迄も無い。即ち受精に際しては水分が絶対に必要な点は水生植物に近く、且つ水分及びそれに溶解している養分は植物体の全面より吸収する型が多い。ごく小形で基物に密着又は這い、群生しているため降雨に際しては殆ど水生植物的な環境下にあると言うことが出来る。従つて基物の水溶性物質、pH などの影響は非常に強く、このことは筆者等の研究から実際に確認される。蛇紋岩地、石灰岩地、花崗岩地蘚苔類フロラの差異は主として之に起因する。

蛇 紋 岩 苔 類

アポイ山の蛇紋岩上に見出された苔類は次の 27 種であった。このうち星印 (*) を附した 11 種はアポイ山及びその近接地 (襟裳岬、猿留、豊似沼附近、幌泉、様似、ピンネシリ、エサマンベツ等を含む) に於て、他の基物上では採集されなかつたものである。

I. Hepaticae occurring on serpentine: (1)* *Barbilophozia barbata*, (2) *Bazzania trilobata*,

(3) *B. ovifolia* var. *vastifolia*, (4) *Blepharostoma minns*, (5) *Bl. trichophyllum*, (6) **Cololejeunea nakajimae*, (7) **C. rupicola*, (8) *Diplophyllum taxifolium*, (9) **Euosmolejeunea auriculata*, (10) **E. obtusifolia*, (11) **Frullania densiloba*, (12) *Fr. jackii*, (13) *Fr. moniliata* subsp. *obscura*, (14) *Herberta sakuraii*, (15) *Jamesoniella autumnalis*, (16) **Jubula hutchinsiae* subsp. *japonica*, (17) *Lejeunea rotundistipula*, (18) *Macrodiplrophyllum plicatum*, (19) *Metzgeria conjugata* var. *japonica*, (20) **Nipponolejeunea pilifera*, (21) *Plagiochila satoi*, (22) *Plectocolea prostrata*, (23) **Radula amentulosa*, (24) **R. boryana*, (25) **R. kanemaru*, (26) *R. obtusiloba*, (27) *Trichocoleopsis sacculata*.

So far as the write has been able to ascertain, the species marked with an asterisk (*) have not been collected on any substrata other than serpentine in the present area.

(1) は日本新記録の極北要素で、欧米では色々な基岩に着生し、その pH は 5.8~6.5 であるが、分布南限の当地域では蛇紋岩上に発見され、pH 6.5~7 であつた。一般に植物はその分布周辺地帯では或る特定の基岩乃至は環境条件下に局限される傾向があり (例えばは欧州のブナが石灰岩地に)、蘚苔類でも多くの例が知られているが、例えば熊本県人吉近郊の石灰岩地に南方系の数種が不連続的に分布している。

(24) は海馬島、本州、四国、九州の高山及び不連続的に Fiji 島に分布する。その他の星印 9 種はすべて日本列島 (乃至近接地域) に分布し、而も (16) 以外は殆ど東北地方以北には未記録の種に属する。即ち本州中~南部からアポイ山蛇紋岩地に不連続的に分布して北限をなす (未発表ではあるが、岩手県早池峯や北海道夕張岳の蛇紋岩地

* 文部省科学研究費に依る研究の一部である。記して謝意を表する。 ** 財団法人服部植物研究所。 *** 同氏の援助協力に対して感謝の意を表する。

に産するデータを持つているが、かかる蛇紋岩地を除外)

以上星印の 11 種のすべてが他地域では決して蛇紋岩地に限られず、否、蛇紋岩上に産する確実な記録は皆無に近く(之は蛇紋岩地賦が少く、且つ余り注意されなかつたためであろう)——他岩石(但し石灰岩を除く)、例えば花崗岩、砂岩、頁岩など、樹皮上、まれに他蘚苔、シダ類などの葉上に生育する。分布中心地(この場合日本中〜南部)に於ては特に然りで、むしろ着生植物と見られるものが少なくない。

星印の無い 16 種のうち *Trichocoleopsis sacculata* など 3 種(北海道新記録)を除いた残りの 13 種は日本北部に普通に産し、分布域の出現頻度も高く、多様な基物(但し石灰岩を除く)に着生する適応度の大きな種に属する。但し蛇紋岩地に優勢な種を挙げると、(8), (10), (13), (18), (26) などで、(2), (14), (21), (23) などが之に次ぐ。この中より当地域や早池峯などの蛇紋岩地の標徴種を採れば (10) *Euosmolejeunea obtusifolia* であろう。

石灰岩苔類

アホイ山西北側のメナシエサンペノに小規模な石灰岩の露頭(高度 250 m)がある。この石灰岩上に採集された苔類は次の 14 種であつた。

II. Hepaticae occurring on limestone: (1) **Acrobolbus mayebarae*, (2) **Athalamia* sp. (sterile), (3) *Conocephalum conicum*, (4) *Frullania truncatifolia*, (5) *Mannia longiseta*? (sterile), (6) *Metzgeria pubescens*, (7) **Plagiochasma intermedium*? (sterile), (8) *Plagiochila ovalifolia*, (9) **Porella gracillima*, (10) *P. grandiloba*, (11) **P. setigera*, (12) *Reboulia hemisphaerica*, (13) **Solenostoma exsertifolium*, (14) *S. triste*.

So far as the writer has been able to ascertain, the species marked with an asterisk (*) have not been collected on any substrata other than limestone.

このうち星印を附した 6 種は当地域では石灰岩以外には見られなかつたもので、残りの 8 種は他の着生基物(蛇紋岩を除く)にも生ずる。前 6 種は全部好石灰岩苔類と呼び得るもので、筆者の調

べた範囲では *Porella setigera* 以外の種は何れも石灰岩上のみに知られる。然し“好石灰岩”と云つても積極的に好むと言うより寧ろ消極的に石灰岩に耐え得ると言うのが真相に近い場合が多い。自然状態では石灰岩地のみに見られる *Plagiochasma intermedium* その他数種を素焼の鉢に栽培しているが、弱酸性の土でも十分に成育する。

星印の無い 8 種中、(6), (10), (14) 好石灰岩苔類と言えるが、他は寧ろ適応度の高い普通種と見る可きものである。日本石灰岩地の標徴種として (1) *Acrobolbus mayebarae* 乃至 (7) *Plagiochasma intermedium* が強げられるが、特に北日本(又は高地)石灰岩地のそれとして 1 種を挙げれば (9) *Porella gracillima* となる。

蛇紋岩苔類と石灰岩苔類との比較

上述の蛇紋岩地産苔類は真の好蛇紋岩苔類と見ることは困難である。第一に蛇紋岩上にのみ産する種は全く存在しない。之に対して石灰岩地産苔類には石灰岩以外には産しない種が少なくない。勿論何れの場合でも生物要因や気候要因を無視することは出来ない。前述の如く、石灰岩地特産種でも、他の基岩上では他の植物との競争に破れて絶滅したが、耐石灰岩性が強いため、石灰岩地のみに遺存的に分布するに至つたと解釈すべきものが多いのである。然しながら蛇紋岩地産苔類と比較した場合、その差は誠に顕著であり、耐蛇紋岩苔類に対照して好石灰岩苔類と言う風な表現が充分許されると考える。

次に蛇紋岩苔類は他の基物にもよく着生するが石灰岩上には見出されず、一方石灰岩苔類は蛇紋岩上には確認出来なかつたことは注目に値するが、この原因として蛇紋岩がマグネシウムを多量に含んでいること(MgO 約 30%, CaO 約 5%), 他方石灰岩がカルシウムを多量に含んでいること(CaO 約 40%, MgO 約 5%)を特に強調したい、この両者の基岩作用は生理的に異質のものであり、それ故上述の如く蛇紋岩と石灰岩両者に耐(乃至好)性の苔類が見出されなかつたと考える****。

**** 井上浩氏(未発表)に依れば好石灰岩苔類 *Porella stephaniana* 外 1 種が蛇紋岩上に各一例確認された由。

苔類フロラの特徴及び高等植物フロラとの比較

高等植物に就てはアポイ山のフロラは先ず館脇操博士、次いで故中井猛之進先生、原寛博士の詳細な研究に依て明らかである。次に蛇紋岩のみに限定せず、全アポイ地域に就て、筆者の調査した苔類フロラの特徴を挙げる。

(1) 北海道の他の産地に比較して種の数は大して差がないが、その発生状況は比較的貧弱である。(2) 即ち当地域は耐蛇紋岩性の苔類と着生基物の pH が弱酸性〜アルカリ性の苔類が主であつて、耐蛇紋岩性の苔類には適応度の高い北海道の普通種以外に、不連続的に当地を分布北限とする南方系苔類の遺存が著しい点は前述の如くである。(3) 反面、当地域に見られない種は着生基物の pH が酸性の苔類及び厳密な意味での高地性の苔類である。前者の例としては *Marsupella* 属、*Scapania* の諸種、後者の例としては *Gymnomitrium*, *Anthelia* の諸属、*Ptilidium californicum* (北海道の高山、未発表) など、両者共に多数挙げることが出来る。(4) アポイの高等植物フロラは、ハイマツが海拔 300 m あたり迄産するなど高地植物が低所に下ることが知られているが、苔類に於てはこの点不顕著である。(5) 不連続分布〜遺存種の多い点は高等植物と同様であるが、前者に於ては

北方系が遙かに多いのに対し、苔類では既述の如く一例に過ぎず、却つて日本中〜南部に分布する種が不連続的に当地域に出現する例が多い。(6) アポイ山では垂直分布が乱れ北方系と南方系とがしばしば同高度に共存する点は高等植物と同様である。然し苔類に於ては相当数の南方系が頂上部迄産するため、この点更に著しいものがある。例えば *Frullania densiloba*, *Cololejeunea nakajimae*, *C. rupicola* などが山頂部に産するが、ここには *Barbilophozia barbata*, *Macrodiplophyllum plicatum*, *Nipponolejeunea subalpina* などの北方〜高地性の種が生育している。又他地域では明瞭に住み分ける *Blepharostoma* 2 種を例にとると、*Bl. minus* に山足から山頂にかけて産するが、上部では *Bl. trichophyllum* が共存する。(7) 高等植物では種の数が近隣地域に比較して多いと言うが、この点苔類では先に記述した如くそれ程著しくない。

以上、高等植物フロラと比較して若干の異同を見出したが、之は苔類の起源〜分化が高等植物より遙かに古く、反面新世代以降の分化、発達が遙かに小と考えられる点、更に既述の如き苔類の生理、生態の特殊性からして、むしろ当然の帰結であらう。蘚苔類フロラに対し植物地理学者並びに生態学者の関心を切望してこの小篇を終る。

Summary

Mt. Apoi is situated in the island of Hokkaidō, ca. 42°N. Lat., 143° E. Long. It consists mostly of serpentine, attaining an altitude of 810 m. above sea level. As shown in the list I, twenty-seven species were found on serpentine, in which eleven species marked with an asterisk (*) are confined to serpentine and the remaining also occur on various substrata other than limestone. In these eleven species, only *Barbilophozia barbata* belongs to the holarctic element. The others are mostly endemic in Japan and her adjacent regions and considered to be of southern origin, the center of their distributional area being generally in Middle to Southern Japan, where they occur on the bark of trees and shrubs and various rocks other than limestone. Thus there are no essential serpentine-loving hepaticae so far as the present area are concerned.

At Esamanbetsu north-west of Mt. Apoi, an outcrop of limestone is found on a small scale. Here fourteen species were collected on limestone, as shown in the list II, in which six species are confined to limestone. These six species are endemic in Japan and its neighbouring territories, where they occur almost exclusively on lime-

stone. Thus they may be regarded as calciphile hepaticae.

It may be a most remarkable fact that few or no hepaticae can occur on both serpentine and limestone rocks, so far as the writer's knowledge is concerned. Generally, hepaticae are hardly tolerant to a certain degree of magnesium as well as excess of calcium in the substrata. The injurious effect of the two rocks is not the same physiologically.

キイチゴ属雑種の研究 III カデイチゴ♀ × ナガバキイチゴ♂について

神 野 太 郎*

Taro JINNO: The Study on the Hybrids in *Rubus* III.

R. trifidus Thunb. ♀ × *R. palmatoides* O. Kuntze ♂

1955 年 9 月 1 日受付

緒 論

筆者は既に *R. trifidus* (♀) × *R. hirsutus* (♂) 及び *R. trifidus* (♀) × *R. ribisoides* (♂) の雑種について報告したが^{2,3)} 引続き今回は *R. trifidus* (♀) × *R. palmatoides* (♂) の雑種について報告する。この交雑試験は 1951 年に行いその結果 2, 3 の雑種を育成することを得、これ等が 1953 年に開花した。

この交雑に用いた *R. trifidus* は松山市近郊で栽培されていたものであり *R. palmatoides* は松山市近辺に自生していたものである。この研究に用いられた両親及び雑種の栽培場所、減数分裂の観察及び花粉粒の測定等の方法は第 I 報及び第 II 報と同じである

観 察

この雑種 F₁ の形態を両親植物と比較したのが第 1 表である。筆者が既に第 I 報及び第 II 報で報告したのと同じく父本に似る場合、母本に似

る場合及び父本と母本の間間型をしめす場合がある (第 1 表参照)。

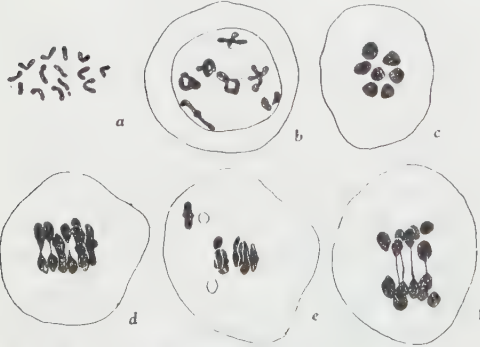
両親植物である *R. trifidus* 及び *R. palmatoides* は染色体数が何れも $2n=14$ であり⁴⁾ 後者には附随体をもつ一對の染色体がある (神野未発表)。この F₁ の染色体は根端細胞で $2n=14$ であり、この染色体中には附随体をもつ 1 個の染色体が観察される。

この雑種 F₁ の花粉母細胞における減数分裂を観察するに Diakinesis 期においては第 1 図 b でみるごとく環状或は X 状に結合した 2 価染色体が観察される。この場合多くは 2 価染色体のみが 7 個現れ、1 価染色体の現れる場合は少い。減数第 1 分裂中期における染色体の対合状態をみるに染色体がそれぞれ対合して 7 個の 2 価染色体を形成する場合が多い (第 1 図 c. d)。7 個の 2 価染色体が形成された場合において、それぞれ対合する染色体間に結合の強固なものや緩いものがあり、後者の場合は核板により多少の差はあるが多くの場合 1 個又は 2 個である。第 1 分裂中期の核板に時々 2 個の 1 価染色体が現れることがある (第 1 図 e)。

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第 1 表 雑種 F₁ 及び両親の形質比較

部分	項 目	<i>R. trifidus</i>	F ₁ -Hybrid	<i>R. palmatoides</i>
花	花 序	聚 繖 花 序	単 頂 花 序	単 頂 花 序
	花 梗 の 方 向	直 立	彎 曲 下 垂	下垂又は斜下垂
	花 梗 の 色	緑	赤	赤
	花 梗 の 毛	少	多	少
	花 弁 の 皺・欠 刻	有	無	無
	雄 蕊 の 方 向	放 射 状	中 間 型	中央に集結
	雄 蕊 の 数	約 110 本	約 95 本	約 75 本
葉	葉 身 の 形	7 深裂又は 5 深 2 浅裂掌状単葉	5 深裂~3 深裂 中葉片稍々大	3 深裂又は 3 深 2 浅裂、中葉片大
	葉 基 脚	心 臓 型	浅心臟又は截型	浅心臟又は截型
	鋸 歯	重 鋸 歯	中 間 型	鋭 鋸 歯
	葉柄・葉脈上の棘	無	無 (稀にあり)	有
	托 葉	長橢円形欠刻あり	狭 長・全 縁	狭 長・全 縁
	茎の太さ (直径)	約 2.5 cm	約 2.5 cm	約 1.3 cm
	棘	無	無 (稀にあり)	有
茎	腺 毛	有	無	無
	色	若い茎—緑 古い茎—赤褐	若い茎—緑 古い茎—幾分赤褐	緑
果実	小 核 の 小 凹 点	有	有	無
	白 毛	無	有	有
	果 托	革 質 で 平 か	肉 質 で 反 卷	革 質 で 平 又 は 反 卷



第 1 図 F₁ 雑種の花粉母細胞における減数分裂及び根端における染色体
a 根端細胞の染色体, b~f 花粉母細胞における減数分裂. d Diakinesis 期, c, d, e 第 1 分裂中期 f 第 1 分裂後期. 白●1 価染色体をしめす.
a ×2600, b~f ×2900

この場合それぞれの 1 価染色体は赤道面の両側に対称的に位置する場合が多い。減数第 1 分裂後期においては赤道面で分裂した染色体が両極に向つて移行しはじめた後、しばしば対合していた染色体が互に細い糸状のものでつながっているのが観察される (第 1 図, f・写真 d)。減数第 2 分裂及び 4 分子形成過程は正常であり別に異常なる現象は認められない。

この雑種及び両親植物の花粉粒はいづれも内容の空虚なものは少くて、内容の充実している花粉粒は第 2 表で見る如く三ついずれも 90%以上をつめている。しかし F₁ の花粉充実度は両親に比して幾分低い (第 2 表参照)。次にこれら三者の花粉粒の大きさについて比較すると第 3 表で見る如く、この F₁ の花粉粒の大きさは両親の何れよ

第 2 表 花粉粒の充実度

要項	植物名	<i>R. trifidus</i>	<i>F₁-Hybrid</i>	<i>R. palmatoides</i>
内容充実している花粉数		876	585	907
同 上 百 分 率		97.6%	91.0%	97.5%
内容空虚なる花粉数		21	58	23
同 上 百 分 率		2.4%	9.0%	2.5%
花 粉 数 合 計		897	643	930

第 3 表 花粉粒の大きさ

植物名	24 μ 以下	24.1-26 μ	26.1-28 μ	28.1-30 μ	30.1-32 μ	32.1-34 μ	34.1-36 μ	36.1-38 μ	38.1-40 μ	40.1-42 μ	42.1-44 μ	44.1-46 μ	合計
<i>R. trifidus</i>		3	5	31	70	16	14						139
<i>F₁-Hybrid</i>	2	6	1	2	9	9	24	22	17	35	7	2	136
<i>R. palmatoides</i>			1	0	1	1	5	10	28	71	26	10	153



第 2 図

a. 花の比較 左 *R. palmatoides*. 中 *F₁-Hybrid*. 右 *R. trifidus*. b. 葉の比較 左 *R. trifidus*. 中上下 *F₁-Hybrid*. 右 *R. palmatoides*. c. *F₁-Hybrid* の花の着方. d. *F₁-Hybrid* の花粉母細胞における減数第 1 分裂後期.

りも変異の巾が広く 24 μ より 46 μ の間にわたつて現れる。又両親植物では花粉粒の大きさの変異曲線は何れも単頂のノルマルカーブをしめすが、この雑種は多頂で複雑である。

この *F₁* 雑種は種子及び果実を形成するが、稔性は両親に比較すると低い。*F₁* 及び両親の自然における稔性率をしめしたのが第 4 表であるが、この表で見る如く *F₁* の稔性率は両親に比較して半分以下である。筆者が現在迄に調査した範囲内ではこの *F₁* の果実で最も高い稔性率をしめしたものが 53% であり、これは両親植物のほぼ平均稔性率に当る。又一果実を形成する小核数も *F₁* では両親に比較して少い (第 4 表参照)。

論 議

この雑種及び両親植物は染色体数は何れも $2n=14$ である。何れも染色体が小さいため核型を用にすることは出来なかつたが、附随体を持つ染色体が父本に一对、*F₁* に一個存在する。この *F₁* の附随体をもつ染色体は父本より来たものと思れ、この雑種の染色体はおそらく両親植物の配偶子のもつ染色体の和で構成されたものであろう。

この雑種における P. M. C. の減数分裂を見ると Diakinesis 期では多くの場合全部の染色体が 2 価を形成するが、第 1 分裂中期核板においては時々

第 4 表 稔 性

植物名	要項	平均稔性率	最高稔性率	一果における 平均小核数	一果における 最高小核数
<i>R. trifidus</i>		68%	94%	91	136
<i>F₁-Hybrid</i>		24%	53%	26	80
<i>R. palmatoides</i>		50%	85%	62	135

1 価染色体が現れる。これは Sharp⁶⁾ の云う如く相同染色体が普通のやうに対合するが、前期の終りまでに分離してしまう Desynapsis に類似の現象であろうと思われる。この場合 1 価染色体が 2 個現れることが多く、しかもこの 1 価染色体が赤道板を中心にして両側に対象的に現れることが多い。これは相同染色体の分離の時期が、前期の終り中期に入る前か或は中期の初期に行われるからだと思れる。

筆者が既に報告した *R. trifidus* × *R. hirsutus* 及び *R. trifidus* × *R. ribisoideus* の雑種では P. M. C. の中期核板で 1 価染色体が 2~4 個或はそれ以上 at random に現れる場合が多かつたが、この雑種では総ての染色体がそれぞれ対合して 2 価を形成するが多い。これはこの *F₁* を導いた *R. trifidus* と *R. palmatoides* のゲノム間の親和性が上述の場合に比して高いからだと考えられる。

この *F₁* の花粉粒は内容充実しているものが 91 % で筆者が既に報告した前述の 2 種の雑種に比較

すれば遙に良好である。これはこの *F₁* の減数分裂過程に余り異常がないからであらう。しかしこの雑種の花粉粒の大きさが両親に比較して変異の巾が広く、しかも変異曲線が多頂で複雑であるのは、この *F₁* がそれぞれ本質的に花粉粒の大きさの異なる両親の交雑によつて導かれたものであるからと思われる。

両親植物の分類学的位置は Koidzumi⁵⁾ によると *R. trifidus* は Subgenus *Anopleobatus* Focke に、*R. palmatoides* は Subgenus *Idaeonius* Focke に属する。筆者は既に第 1 報でこの二つの Subgenus 間の種間雑種における不稔性の場合を報告したが、この *F₁* 雑種は稔性である。即ちキイチゴ属では異つた Subgenus 間の種間雑種でも用いる種によつて不稔性の場合或は稔性の場合が生ずるものと考えられる。

最後にあたり種々御指導を頂いた下斗米教授に原く感謝の意を表する。

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Résumé

1. The results of the research on the fertility, characteristics and pairing of the chromosomes in the reduction division in P. M. C. of the *F₁*-hybrid which is raised artificially by crossing *R. trifidus* (♀) and *R. palmatoides* (♂) are given.

2. The chromosomes at the metaphase I in P. M. C. pair well but one or two pairs are loose in the union of chromosomes.

3. The pollen fertility of the *F₁*-hybrid is rather high, namely the rate of the pollen having contents is 91%.

4. The *F₁*-hybrid is fertile but the average percentage of the fertility in the nature (24%) is lower than those of the parents (65% and 50%).

第20回大会講演

特別講演

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木原 均：カラコラム・ヒンズークシ学
術探検報告

一般講演

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葉枕について
下瀬 敏，斎藤真太郎：蘚類の蒴歯の発生学的研究 IV ウミバユスサケの蒴歯の発生について
肥田美知子：球果の発育過程から見たメタセコイア及びその近縁種の類縁関係
引田 茂：葉の構造から見た *Metasequoia* の類縁関係について
二宮淳一郎：メタセコイア幼植物の生育について
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新崎盛敏，野沢治治：秋野喜一：緑藻カサノリ族の体形成
伊藤 道夫：モエジマシダ前葉体の成長について
高橋 千裕：無孢子生殖的に再生させたシダ配偶体について
北村 玲子：コウヤマキの葉における異型細胞の発生について (続報)
原 真：斑葉の研究特に細胞間隙による斑葉について
沢田 武男：シダモク (?) に関する観察及び胚発生
黒木宗尙：アマノリ類の生活史の研究 (続報)
福島 博：氷雪藻類に関する新知見
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川崎 次男：シダの胞子の発芽能力
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相見雲三, *村上 高: 登熟機構に関する細胞生理的研究 1, 種子の発育過程におけるアミラーゼ, フォスフォリラーゼ, 磷酸等の変化

村上 進: ユリ科植物の炭水化物の研究 (第 2 報) アマドコロの炭水化物について

安村 明, 入来義彦, 三輪知雄: イネ・コムギの結実期中における炭水化物の消長

入来義彦, 三輪知雄: *Siphonales* 緑藻の炭水化物に関する新知見

大槻虎男, 今井百合江子: 糸状菌による蒟蒻マンナーゼ産生について

大槻虎男: コニシリン生産菌培養における蒟蒻マンナン添加について

本 会 記 事

第 20 回 大 会

昭和 30 年 10 月 12 日 (水)~16 日 (日) の 5 日間にわたって広島で開催された。前半 3 日間が講演、総会、懇親会等で、後半は見学であつた。好天に恵まれて参集会員 381 名の外、会員外の 120 名余の聴者もあり、非常に盛会であつた。一般講演は申込数 238 という多数にのぼつたので 4 会場、即ち A 教育学部講堂、B 文学部 212 号講義室、C 文学部 9 号講義室、D 文学部 7 号講義室、が用いられた。講演時間は一人 8 分~15 分で、どの会場でも終始活潑な質疑応答が交わされ、欠席などにより結局 225 の講演が行われた。一般講演を第 2 日で終り、第 3 日の午前中に特別講演、総会がすみ、ついで農協ビルで懇親会が開かれた。14 時懇親会場に各見学班のバスが出迎え夫々出発した。なお第 2 日の午前の講演が終つて A 会場の裏に用意されたスタンドで記念撮影が行われた。

総 会

10 月 14 日 10 時 40 分から教育学部講堂で、特別講演に引続いて行われた。服部会長挨拶の後、会長司会のもとに、予め準備されたチャート

に従つて幹事長が報告、説明し、次の案件が議せられた。

(1) 会長、評議員の改選結果の報告 (30 年 4 月号掲載)。幹事長、幹事 (4 月号)、編集委員 (5 月号) の新任の報告ならびに承認。

(2) 現在会員 (30 年 9 月 30 日現在) 1061、内名誉会員 8、特別会員 26、外国通信会員 4、終身会員 49、通常会員 974。会員移動 (29 年 10 月 1 日~30 年 9 月 30 日)、死亡 5、退会 6、除名 83、入会 146。

(3) 雑誌の寄贈、交換。寄贈：発送したもの国外 2、国内 2、受理したもの国外 5、国内 29。交換：発送したもの国外 77、国内 34、受理したもの国外 80、国内 35、予約購読 167、その他、発送 3、受理 1。

(4) 昭和 29 年度決算報告 (30 年 6 月号) があり、承認。

(5) 昭和 31 年度予算ならびに会費値上げの件、まず現在の会費 600 円にもとづく予算案と、評議員会原案の 900 円案をならべて提出。600 円案では 1 冊 30 頁、年 10 回の雑誌がやつと出せる程度で文部省の刊行補助費に対する要求の年 450 頁には非常に遠く、また手持原稿などからみても評議員会案の会費 900 円、44 頁 10 回が望

昭和 31 年度 (昭和 31 年 1 月~12 月) 予算

収 入 (単位千円)			支 出 (単位千円)	
会 費	900	(会 費)	出 版 費	900(44頁10回)
予 約 購 読 料	221	(900円)	発 送 費	100
一 部 売	24		編集関係費	87
バックナンバー売上金	30		図書関係費	50
広 告 料	50		庶務関係費	150(選挙、名簿準備金を含む)
利 子	3		大会関係費	80
文 部 省 刊 行 補 助	200		支部補助	10
			幹事手当	84
			予 備 費	12
小 計	1,428		小 計	1,428
前 年 度 繰 越 金	74		次年度繰越金	74
総 計	1,502		総 計	1,502

ましいことが説明され、さらに会費 1000 円案について補足説明があつた。会費値上げにともなう会員の減少、文部省の刊行補助費の削減についての質疑など、極めて熱心に討議が続いたが、最後に挙手によつて決をとつた。原案（会費 900 円案）賛成者 134、不賛成 11（内 1000 円案賛成 6）、いずれにも挙手せぬ者 2 で評議員会原案が可決され、31 年度予算が表の如くきまつた。

(6) 会則変更が以上会費の値上げなどにもない上程され、次の如くそれぞれ可決された。

会則第 13 条 本会の会計年度は 1 月に始まり 12 月に終る。

付則第 1 第 1 条 通常会員の会費は年 900 円とし 300 円ずつ分納することもできる。終身会費は 15,000 円とする。

このほか国外在住会費に限り植物学雑誌の送料を負担する。

同 第 2 条 評議員、編集委員以外の役員は在住中会費を要しない。

懇親会開始の予定時刻も過ぎたので、その他の事項（評議員会記事を参照された。）はその都度誌上に発表するとして 12 時 40 分を閉会した。

評議員会

10 月 11 日 16 時—12 時、教育学部会議室で開催。出席者は評議員 15 名（欠席 7 名）、会長、幹事長、幹事 5 名。まず服部会長から就任の挨拶があり、つづいて大会会長に下斗米直昌氏が推薦され、次の諸事項が協議された。

(1) 評議員、会長の改選結果報告。幹事長、幹事、編集委員の新任の報告および承認。なお編集委員の任務、選出方法、謝礼（1 編 300 円程度）、会費の免除の中止などについても協議した。

(2) 特別会員は現在相当数あり、また 75 周年も間近かなので、本年は推薦をみちわせた。

(3) 会員の移動報告とともに、29 年度までの会費未納者は、文部省への報告などの関係もあつて除名したが、これは会費の納付あり次第会員に復帰することの説明があつた。これに対して評議員より、支部に連絡してもらえば本人に会費納入をすすめるとの話があつた。

(4) 雑誌交換の報告があり、つづいて学会の所蔵図書が膨大になり整理しかねており、不用と

思われるものは前当に処分することを認めた。また図書によつては支部に保管を依頼した方がよいとの話もあり、図書幹事がリストを作つて支部に問合せることとした。

(5) 昭和 29 年度決算報告があり承認された。文部省刊行費補助費は 21 万円と決定したが、これには論文 450 頁以上を出版する要求がある旨報告があつた。

(6) 昭和 31 年度予算について会費 600 円、800 円、900 円 1000 円の案を示して説明があり討議に入つた、先の文部省の要求もあり、現在の会費ではこの 12 月末では残金 3 万円程度となり会の運営が困難である旨の報告があり、また受理原稿が 30 余編あり、この会員の要望に答へるにも現在の 30 頁立てよりも増頁する必要があると、必然的に会費値上げが上程された。すなはち、800、900、1000 円の各案について、かなりつつこんだ討議がかわされたが、評議員会としては 900 円案を最良として、さらに総会に諮ることとなつた。また終身会費についても種々論議があつて 15,000 円に決定した。なおこれにともない編集委員、幹事、幹事長などの会費の免除中止事務員の雇入れなどについても提案あり論じられた。

(7) 会計年度の変更は昨年からの宿題となつていたが、会費の納入状況などより、会誌の年度と合せて 1 月—12 月に改めることとした。

(8) 会則の変更が以上の決定に伴つておこるので、その原案を提出し可決した（総会記事参照）。

(9) 三井信託予金 15,000 円がふるくから学会基金としてあるが、現状にそぐわぬので通常会計に入れる。

(10) 各種の奨励金候補研究の推薦依頼があるが、支部に問合せも提出される研究が極めて少なく、推薦しても効果も期待されないの、特に学会としての推薦手続をすることは止めて、植物学雑誌に案内を載せる程度にとどめることとした。

(11) 第 21 回大会は前々から定つていた北海道で行うことが確認された。松浦一氏から、その大会々長に山田幸男氏を推薦したい、また特別講演を会長に依頼したいとの発言があつた。

(12) 小倉謙教授記念出版については色々と記

記念会からの要望ももつたが、学会として行記念会からの多少の補助をえて 2 号分を合併して 100 頁前後の記念号を出すことを請つた。

(13) 植物学学術用語の選定について服部会長から説明があり、それに対する協力が希望された。

(14) 75 周年記念事業についての近藤武夫氏の手紙が熊沢正夫氏から披露された。

懇 親 会

10 月 14 日、総会終了後直ちに 2 台の大型バスで程遠からぬ農協ビルに運ばれ午餐の懇親会が開かれた。下斗米大会々長、服部学会々長の挨拶に始まり、各地区を代表するスピーカーが指名された。早朝からの雨も降り止んで 14 時出発の見学は各班とも実施されることになり、13 時 50 分酩酊酒茂鶴も残り多いまゝになごやかな会は閉ぢられた。

公 開 講 演

10 月 15 日午後広島市と福山市との 2 ケ所で開催。広島市では 14 時から广大教育学部講堂で、奥野春雄（電子顕微鏡と生物学）、館脇操（北の植物）、坂村徹（植物生理学の進歩と応用）3 氏の講演並びに映画（原子力の利用）があり、参会者 300 名余で、18 時盛会のうちに終つた。福山市では 13 時から市立旭小学校講堂で、猪野俊平（植物学の人生への貢献）、芦田譲治（生命と自然科学）両氏の講演が行われ、300 余名の聴講者が集り、これまた当地では稀に見る盛会でもつた。

関係諸学会

1) 形態学細胞学談話会 10 月 13 日、17 時～21 時、広島大学本部第一会議室、71 名、下斗米直昌氏の挨拶、会食後、篠達喜人氏を座長として今大会の研究発表に関連した話題提供が次の 3 氏によつてなされ、活潑な討論がされた。先づ熊沢正夫氏が分枝の機構について主筆の役割を論じ、次いで新家浪雄氏が体細胞分裂の生理学的相異点を論じ、最後に下斗米直昌氏が鮮やかな顕微鏡写真を幻燈で示しながら種の系統を研究する場合における新核について論じた。終りに篠達氏から核型記号の統一について提案があつた。本談話会をこの様な形式で行つたことは新しい試みとし

て皆に親理された。

2) 第 8 回植物分類学会、10 月 12 日、18 時～22 時、教育学部会議室、63 名、会食、大会開催地の幹事から今年度から植物学会々員であれば誰でも入会出来る様になつた経緯や抱負が述べられた。次年度の幹事として印東弘玄（庶務）、加崎英男（会計）の両氏が推され、次回大会開催地の幹事は北大から推薦することとなつた。津山尙氏から隠花植物科名の統一について報告並びに提案があり、既存の小委員会に一任された。Taxon の購読料の負担は当分現状のままとした。本田正次氏から「絶滅しつつある植物目録資料の提出」について国際自然保護連盟からの依頼、応答すべき植物目録の原案について説明および追加記入の要望があつた。協議が終つて北村四郎氏のアフガニスタン探検談並びに山田幸男氏の欧洲視察談が、いづれも美事な天然色幻燈で繰広げられた。

3) 植物生態学会談話会、10 月 13 日、18 時～22 時、教育学部会議室、77 名、会食、堀川芳雄氏の歓迎挨拶に続いて自己紹介に移つた。次いで館脇操（次回大会開催地北海道植物景觀）、細川隆英（着生植物群落の経統観察）両氏の天然色幻燈を用いた講演があり、質疑応答が交わされた。終つて懇談に入り、次回大会の見学、次年度生態学会及び本談話会に対する希望、更に共同研究・文献の交換・研究能率の向上等に対して種々有益な意見が開陳された。

4) 植物生理学談話会、今年度は京都大会での要望に依えて 12、13 日の 2 日開催毎 18 時～21 時、A 会場両日とも約 100 名、第 1 日は植物の吸水機構（座長：芦田譲治氏）を主催地研究室（福田八十楠氏）の研究を中心に論議した。第 2 日、原爆と植物被害、藤田哲夫氏の被害植物に現われた変異について説明があつた。形態形成の生理（今村駿一郎氏）。容易に入手でき、生化学的研究材料のとりやすい、生長点の大きい植物が要望せられた。小島均氏により開花素質が原形質の状態変化と解されるものとホルモンが形成されたと思われるものとの 2 種あることが説明された。代謝と酵素。主としてフォスホリラーゼについて相見豊三、小野林両氏の対談、また柴田万年氏の花青素の問題と化学的研究と生理現象との結びつきについての議論があつた。電気生理に

関して渡辺篤、柴岡孝雄、渡辺一郎氏等の話があった。また現在のように大会々場を生理、生化学の2つに分けず、なるべく1会場にしてほしいとの意見もあった。

5) 菌類学会, 10月13日, 18時~21時, 広大理学部長室, 24名, 司会者日野巖氏の菌類学会成立の経緯についての説明に続いて, 中沢亮治氏の“紅酒”に関する興味ある談話が, 暖皮, 古い菌類目録, 菌の交雑, アカパンカビなる日本名の出所, *Actinomyces* を中心とした種の問題等々について談論風発。Micrology の今後の発展についても話が盛えられた。

見 学

各見学班はいずれも10月14日14時懇談会場からバスで出発した。

1) 市内見学班 55名, 雨雲低迷の原爆記念館見学, 約30分, 原爆ドームの前を通つてABCC(原爆傷害調査所)等, その破壊の大要を聞き, 施設の実態を約1時間にわたつて見学。次いで縮景園(泉邸—もと浅野侯の庭園)に入り, 説明をたどつて被爆前の庭園を偲ぶこと約30分, 5時20分広島駅前にて解散。

2) 宮島見学班 36名, 途中原爆ドーム前を通り原爆記念館を見学。雨上りの内海の景色を車窓から望みながら15時30分宮島到着, 貸切の小型船上の人となり10数分で厳島の土を踏む。折よく干潮として途中に聳立する大鳥居の間近にシャッターを切ること頻り, 森安権宮司の案内で先づ宝物殿を拝観, ついで本殿に参拝, 千景園を見学して一応17時過ぎ解散した。更に紅葉谷や大元公園を散策し或は土産物店に珍物奇物を漁つた会員も少なくなかつた。

3) 三反峡班 一行18名は八幡高原・三反峡班と共に出発, 16時10分加計到着, ここで八幡高原班と分れて17時三反峡入口に到着した。奇岩碧水紅葉の絶景の峡谷を溯ること約1時間で宿舎黒淵荘に着く。15日, 冷氣と森閑の夜が

おくれは昨日と違つて變つての晴天, 探勝と植物採集とをかねて絶壁にかゝる山道を進む。対岸の紅葉は覚むるばかりの鮮やかさ, 11時過ぎ三段荘に着く。先づ三段滝を探勝し, ついで昼なを暗い猿飛の岩間を小川に乗つて進み, 二段滝の飛沫をあびる。折返して三段荘で昼食, 13時帰路につく。15時30分三段峡入口で乗車, 18時50分広島着解散。

4) 八幡高原・三段峡班 懇親会の雰囲気そのままバスに寄せ, 長行6時間, バス中でマイクを奪い合いながら中国脊梁奥深く進む。19時高原の日全く沈み, 秋冷の八幡高原に一行14名は村民の心からなる歓迎をうけ, その名も鄙びた蓬屋の山家に広島の疲れをはじめて癒す。明ければ秋晴れの15日, ホクチアザミ・ウメバチソウ・マツムシソウなどの咲乱れる高原を横切つて主山ブナの大原始林に覆かれる刈尾山に向ふ。本田正次氏ははじめ一行元氣極めて旺盛, 八合目附近のバスノハイチゴを観察し, 1223mの頂上をきわめて遙かに見おろす日本海を背にして記念撮影を行ひ且つ祝杯をあげる。再び蓬屋に帰り, 昼食には高原名物のソバに舌鼓を打つ。樽床でワリンゴを賞味し, 奇景龍門では一枚岩の上で心のこもつた八幡村民の酒肴の宴をうけ, ここで別れをつける。ウスギヨウラクの多い三つ滝・三段滝の絶景に心を奪われ, 植物の種類の多いのに驚きながらいつか三段荘の宿舎にたどりつく。夜は峡谷産のイワナに杯の傾きも早く, 堀川芳雄氏の司会で各自30分以上の履歴発表の会が開かれた。半生にわたる波乱万丈の懐旧譚は各人多様多種, 夜の更けるにも気づかず, 人あり, これを称して『三段峡のアラビアンナイトなり』と, 翌16日猿飛・二段滝附近の豊富な植物を探り, 紅葉に映える峡中の山路にクロタビカブラ・マルバフエイチゴなどを採集して峡を出る。ここで高下常一氏に歓待され, 2日間にわたる疲れも霧散してバスに乗る。19時広島帰着, 一同再会を期して解散す。

植物学雑誌バックナンバー在庫表 (昭和 30 年 11 月現在)

全巻在庫するもの

巻	年	号
24	明. 43	276—287
25	明. 44	288—299
32	大. 7	373—384
33	大. 8	385—396
44	昭. 5	517—528
48	昭. 9	565—576
49	昭. 10	577—588
51	昭. 12	601—612
52	昭. 13	613—624
53	昭. 14	625—636
58	昭. 19	685—690
(688—690 合本)		
60	昭. 22	703—714
(全巻合本)		
62	昭. 24	727—738
63	昭. 25	739—750
64	昭. 26	751—762
65	昭. 27	763—774

一部在庫するもの

巻	年	号
6	明. 25	62—70
7	明. 26	72—82
8	明. 27	84—94
9	明. 28	97
10	明. 29	108, 113, 114
14	明. 33	157—164, 166
17	明. 36	194, 197, 198
18	明. 37	206—215
19	明. 38	226
20	明. 39	231, 233—239
21	明. 40	241—251
22	明. 41	252—260, 262
23	明. 42	267—275
26	明. 45	302—212
27	大. 2	316—324
28	大. 3	326—334
29	大. 4	346—348
30	大. 5	350—356, 359
31	大. 6	362—372
34	大. 9	368—404, 407

37	大. 12	438, 439—444 (合本)
38	大. 13	446—452, 455
40	大. 15	472—474, 479
41	昭. 2	487—492
43	昭. 4	506
45	昭. 6	529—531
		533—540
46	昭. 7	543, 544, 547—552
47	昭. 8	554, 555, 558—564
50	昭. 11	590—600
54	昭. 15	947, 648
55	昭. 16	651—653, 957—600
56	昭. 17	661—664, 667—672
59	昭. 21	691—694
61	昭. 23	717—726
66	昭. 28	775—780, 783—784
67	昭. 29	789—794, 297—798

全巻在庫のないもの

巻	年	号
1—5	明. 20—24	1— 58
11	明. 30	119—130
12	明. 31	131—142
13	明. 32	143—154
15	明. 34	167—178
16	明. 35	179—190
35	大. 10	409—420
36	大. 11	421—432
39	大. 14	457—468
42	昭. 3	493—504
57	昭. 18	673—684

以上の通りですが、この外に製本ずみのものなど多少ありますので (例. 1—10 巻), 上の表で在庫なしとなつているものも、まとめて御希望の場合にはおわけ出来ることがあります。特に記してあるものの外は、58 巻まで 1 号 1 冊, 59 巻以降 67 巻までは 2 号で 1 冊となつております。

値段は送料を含めて 1 冊会員 200 円, 非会員 250 円です。ただし 46 巻 544 号 (50 周年記念号) および 51 巻 605, 606 号 (柴田教授記念号) 会員は 500 円, 非会員 650 円です。御注文には必らず巻 号 年を明記して下さい。

日本植物学会会則

(昭和 30 年 10 月 14 日改正)

第 1 条 本会は日本植物学会という。

第 2 条 本会は植物学の進歩と普及を促し、あわせて会員おたがいのしたしみを増すのを目的とする。

第 3 条 本会は前条の目的を達するために「植物学雑誌」そのほかの出版物の刊行、大会・講演会・講習会などの開催、そのほか必要と思われる事業を行う。

第 4 条 本会の会員は次の 5 種とする：

通常会員・終身会員・特別会員・外国通信会員・名誉会員

第 5 条 通常会員とは所定の会費を納めたものをいい、終身会員とは所定の終身会費を納めたものをいう。

第 6 条 特別会員とは引続き本会の会員であつて本会に対して、むしろ、功勞があつた者、外国通信会員とは本会に要するが、外国人、また名誉会員とは植物学に対して功勞があつた者で、いずれも評議員会・協議委員会が総会で推薦し承認された者をいう。但しやむを得ない場合は、あとで総会の承認を求めることがある。これらの会員は会費を要しない。

第 7 条 本会には地方支部を置き、会員は、それかの地方支部に属するものとする。地方支部

についての規定は別に設ける。

第 8 条 本会には次の役員を置く：

会長 1 名・幹事長 1 名・幹事若干名・評議員若干名・編集委員若干名

第 9 条 役員は会員の中から選出し、任期は 2 年とする。但し重任することができる。選出についての規定は別に設ける。

第 10 条 会長は会務の主体をなす。幹事長は会長を助けて会務を処理する。幹事は庶務・会計・編集・図書管理など日常の会務を行う。

第 11 条 評議員は評議員会を構成する。評議員会は会長の諮問の範囲で本会の要務を審議し、また総会への提案を作る。

第 12 条 編集委員は編集委員会を構成する。幹事長はその長となる。編集委員会は「植物学雑誌」の編集に関しての一切の責任を負う。

第 13 条 本会の会計年度は 1 月に始まり 12 月に終る。

第 14 条 本会は原則として毎年 1 回総会を開き、会務を協議し議決する。なお会長が必要と認めた場合には臨時総会を開くことができる。

第 15 条 本会は総会の時大会を開き研究発表などを行う。大会には大会会長そのほか若干名の(裏面へつづく)

きりと線

入会申込書

氏名	男	女	この紙をきりとつて所要の事がらを記入または○でかこみ会費をそえて学会あてにお送りください。どなたでも入会できます。
ふりがな	明治 大正 昭和	年 月 日生	
勤務先 (所在地)			
住所			
通常 終身	会員に	昭和.....年 から支部へ
雑誌の送先を指定してください。希望する方へ○印を			

入会の申込、会費(年 900 円)の払込、そのほか会へのご連絡のあて先は：

東京都文京区東京大学理学部植物学教室内 日本植物学会です。それから会費の払込は 振替貯金口座 東京 11190 番を利用されるのが最も確実ですが封書に現金を封入することも認められていますからこの方法もご便利です。なお振替でお払込の場合は特に領收書をさし上げませんからあしからず。

臨時の役員を置くことができる。大会会長は会長が推薦し、そのほかの役員は大会会長が依頼する。

第 16 条 会員は会合に出席して講演をし議事に参加し、「植物学雑誌」に投稿し、また本会所有の図書を開覧することができる。また毎月無料で「植物学雑誌」の配布を受ける。

第 17 条 会員が退会しようとするときは、そのことを本会に通知しなければならない。もし会費

の滞納があるときはその際全額を納めなければならない。但し既に納めた会費は一切これを返さない。通常会員が会費を滞納したときは直ちに前条の権利を停止し、1 カ年以上滞納したときは除名することがある。

第 18 条 本会の会則または付則を変更するには総会または臨時総会でこれを協議し、出席会員の 3 分の 2 以上の同意を得なければならない。

付則第 1 会 費（会則第 5 条関係）

第 1 条 通常会員の会費は年 900 円とし 300 円ずつ分納することもできる。終身会費は 15,000 円とする。
このほか国外在住会員に限り植物学雑誌の送料

を負担する。
第 2 条 評議員編集委員以外の役員は在任中会費を要しない。

付則第 2 地方支部（会則第 7 条関係）

第 1 条 地方支部は原則として 50 名以上の会員のある地方に置き、北海道・東北・関東・北陸・中部・近畿・中国四国・九州の 8 とする。
第 2 条 会員は居住地の支部に入るのが原則であるが、事情により他の支部に属することもで

きる。
第 3 条 支部には支部長を置く。支部長は支部を代表する。
第 4 条 そのほかの規定は各支部ごとに設ける。

付則第 3 役員の選出方法（会則第 9 条関係）

第 1 条 会長は全会員の投票で選出される。その際評議員会は若干名の候補者を推薦することができる。
第 2 条 評議員は各支部別に支部会員によつて選出される。その定員は各支部最低 2 名とし、会員数が 100 名を越える支部では 50 名まで

とに 1 名を増す。評議員は引続き 3 期選出されることはできない。なお会長および幹事長は評議員を兼任することができない。
第 3 条 幹事長・幹事・編集委員はいずれも会長が指名して総会の承認を認める。

..... き り と り 線

入会や転居などの場合には必ず別に支部へも連絡してください。支部のあて先は次のとおりです。なおどの支部へ入るかは大体地理的にきまるわけですが、ご都合で A 支部よりも B 支部の方が便利だという方は B 支部に入られてもよいことになっています。

- | | |
|--------|------------------------------|
| 北海道支部 | 札幌市北 8 条西 5 丁目 北海道大学理学部植物学教室 |
| 東北支部 | 仙台市片平町 東北大学理学部生物学教室 |
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Electrical Change Caused by Blazing in a Non-sensitive Plant

by Kiyoshi KAWANO*

河野 清：火焰による非感覚植物の電位変動

Received August 23, 1955

It is well known that the transmission of a stimulus in sensitive plants is accompanied by the appearance of an action current. However, only few cases have been reported about the spreading of the action current for non-sensitive plants. Using sweet potato plant, the author observed the transmission of potential changes caused by blazing at a certain part of the plant through petioles and stems. Although several papers about this phenomenon were already presented last year in Japanese an outline of the works is reported here.

The author is indebted to Professor Jôji Asida, Professor Isao Hatakeyama and Mr. Jirô Katô for their constant advice and encouragement.

Material and Method

Sweet potato slips used in the experiments reported here were etiolated ones grown in a darkroom at 25°C. The electrical response of the green plants grown in the field, however, was not of so different a category from that of the etiolated ones in its essential feature.

For the measurement of the electric potential, a kind of Wheatstone bridge was constructed using the vacuum tube UX-54, whose internal resistance and resistance between space charge grid and filament served as the two arms, being combined with a potentiometer at the input side, and the bridge circuit was operated as a zero point instrument. A pair of non-polarizable electrodes used was of a Hg-HgCl-KCl system.¹⁾

Results

The two electrodes were put on a petiole, and the tip of the leaf was exposed to flame for about one second. A typical record of the potential change is illustrated in Fig. 1. A little while after the leaf was flamed, the potential at the distal electrode became strongly negative against the basal one, followed by a recovery and then irregular fluctuations. Unlike the cases of *Mimosa* and animal nerves, the potential change was not diphasic but monophasic.

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In another experiment, the potential difference between petioles of two different leaves was measured, as illustrated in Fig. 2.* The tip of a leaf whose petiole carried an electrode E_1 , was flamed. When the two electrodes were far from each other, the negativity of the stimulated side lasted for a few minutes, as shown in the figure. Similar potential changes were recorded also when electrode E_2 was earthed.

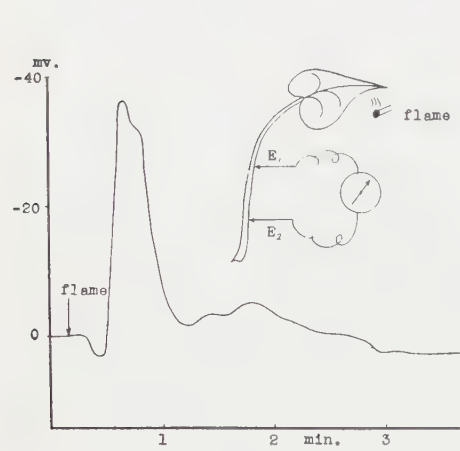


Fig. 1. Potential change at distal electrode, E_1 , against the basal one, E_2 .

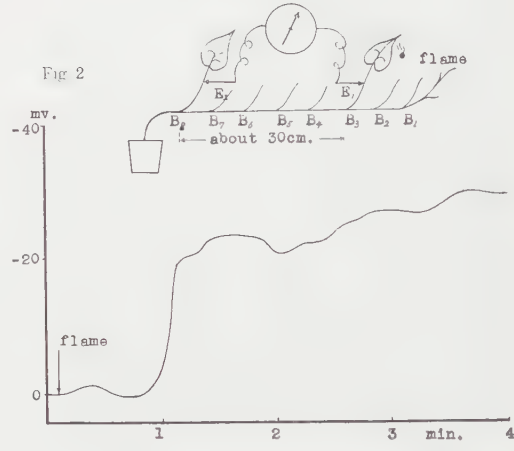


Fig. 2. Potential change at the electrode on the petiole with the blazed leaf against another one on the non-blazed.

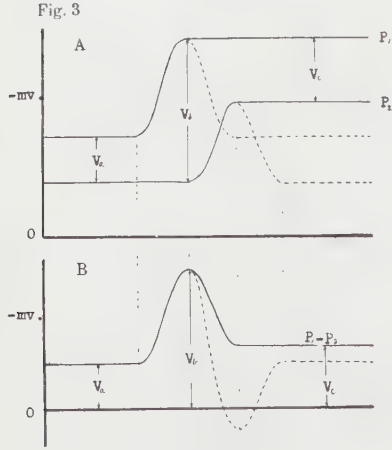


Fig. 3. Explanation of the occurrence of the monophasic response (see details in the text)

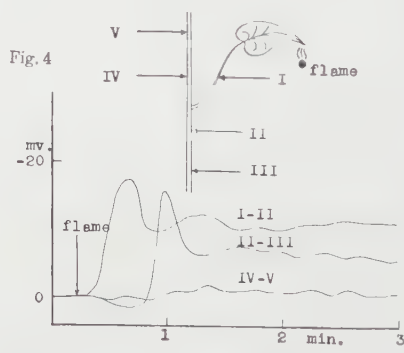


Fig. 4. Various figures of the potential change corresponding to the different positions of the electrodes attached.

* Since immature leaves are folded, the youngest leaf that has been unfolded was denoted as B_1 , older leaves being successively numbered basipetally.

The monophasic nature described above may be accounted for by this persistence of the potential on the stimulated side. Let P_1 represent the potential change set up at the distal electrode on a petiole, and P_2 that at the basal one on the same petiole (Fig. 3, A). Then, P_1 minus P_2 may give a monophasic curve ($V_a - V_b - V_c$ in Fig. 3, B), if the once established potential level be persisted. The broken lines, on the other hand, represent the case when the negative shift of the potential at each electrode might be settled in the original level after some short duration, namely the case of diphasic changes, if it could occur.

In order to see how the effect of blazing is propagated, electrodes were put on various parts the one on the petiole of the blazed leaf and the other on a part of the stem below (I-II, Fig. 4) or above (Fig. 5) the base of the petiole; otherwise both electrodes on the inter node below (II-III, Fig. 4) or above (IV-V, Fig. 4) the flamed leaf. Comparison of the curves, II-III with IV-V in Fig. 4 indicates that the electrical change is induced only in the part of the stem below the flamed leaf, but not in the part above it. And the curves of potential I-II in Fig. 4 and A in Fig. 5, can be also explained by the basipetal propagation of the blazed effect and the absence of the acropetal conductance. As represented by the curve A in Fig. 5 the electrical negativity at the excited site, once established, continues for a few minutes in most cases, followed by the gradual restitution.

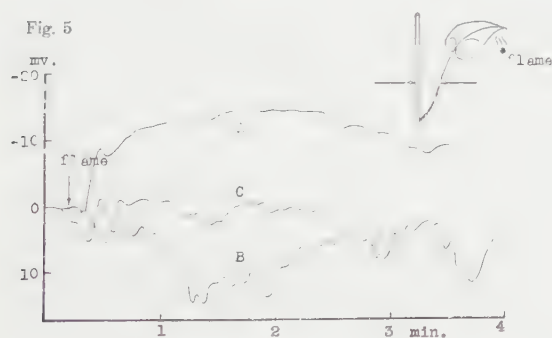


Fig. 5. A: an example of potential change observed after the first flaming,
B: effect of the second blazing 2 hours after the first,
C: effect of the second blazing 24 hours after the first.

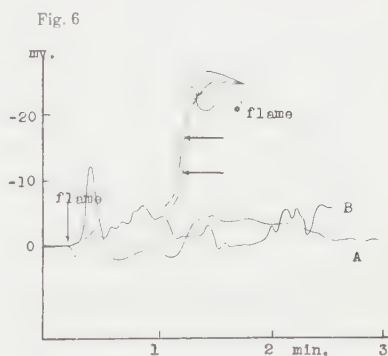


Fig. 6. A: effect of the second blazing 48 hours after the first, B: electrical change with a wilted plant.

When, however, the apical part of the leaf was flamed again, two hours after the first flaming which had caused a potential change as A in Fig. 5, there appeared certain irregular potential changes (B, Fig. 5) in a quite different feature from the preceding response. Some abnormal state must be retained in the plant for a long time after the first blazing, although the electrical potential itself have been settled in the original value within a short time. In order to obtain informations about this persistence of the unhealthy state, the plants once blazed at the leaf tip were kept in a dark room at 25°C and the same leaves were blazed again after 24 and

48 hours respectively. The pattern of electrical change was shown to be still abnormal in the former case (C, Fig. 5), but more or less close by the normal reaction in the latter case (A, Fig. 6). Hence, some several ten hours seem to be needed for the original state to be recovered from the disturbance caused by the blazing. Irregular reactions were observed also when the plant was wilted (B, Fig. 6).

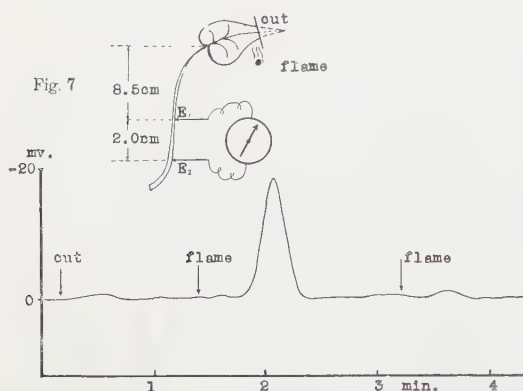


Fig. 7. Absence of the cut effect on the leaf in contrast to the case of flaming.

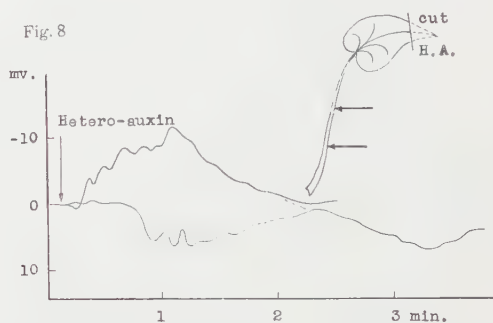


Fig. 8. Two examples of potential change observed after an application of heteroauxin at the cut end of a leaf.

Now, it is a matter of importance to see whether similar electrical effects can be realized also by other kinds of stimulus. Various methods of stimulation were tested for this purpose. At first the leaf tip was cut by scissors or a razor, but no electrical response could be observed in its petiole. The decapitated leaf was found to be responsive when the cut end of the leaf was flamed later (Fig. 7). In further investigations the commercial alternating current of 100 Volts, a concentrated solution of H_2SO_4 , HCl or $CuSO_4$ or boiling water was applied to the midrib of the leaf, but such a clear-cut response in the potential change as realized in the blazing of the leaf tip could never be found in these experiments. But it is to be noted that a potential change appeared on the petiole, when the cut end of a leaf was dipped in a concentrated solution (1 per cent) of heteroauxin (Fig. 8).

Another important fact to be mentioned is that the effect of blazing at the leaf tip did not travel down through the part of the petiole when it was treated by boiling water beforehand or cooled by ice. Similarly, if the leaf tip had been killed by boiling water, blazing of this part did not cause electrical change in the petiole.

Discussion

Although as to the mechanism of propagation of the electrical reaction in the plant tissue we can draw as yet no decisive conclusion, from the results obtained it may be suggested some substantial conception about the nature of the phenomenon, as mentioned in the following. When the leaf tissue is heated by flame, some substance (or substances) may be produced and translocated basipetally through living

cells. The part of the petiole or of the stem affected by the substance may become electrically negative. From the viewpoint of this material translocation theory we can understand most clearly the fact that a duration of more than 24 hours is needed for the affected tissue to recover its normal responsibility to another blazing, while the potential change usually disappears in only a few minutes.

The imazined substance seems to be neither produced by the action of strong mineral acids nor by the treatment with boiling water. Heteroauxin can cause an electrical change, but the process of this change differs from that by the blazing. The relation of the substance here concerned, to those which are considered to cause the leaf movement (2, 3) and electrical change (4, 5) in *Mimosa* is worth while to be studied, because the general excitability of plant cells is considered to be the same in nature in both sensitive and non-sensitive plants.

Summary

With the slip of sweet potato, a "non-sensitive" plant, an electrical potential change was observed to occur at the petiole and stem following after the blazing of the leaf tip. The effect can be propagated only basipetally.

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Studies on Yeasts Isolated from Pine Honey I

Various Observations on *Torulopsis candida* (Saito, Lodder)*

by Minoru YONEYAMA**

米 山 稔: 松蜜から分離された酵母菌の研究 (第一報)
Torulopsis candida (Saito) Lodder に関する二, 三の研究

Received November 9, 1955

A sweet sap exudes from protuberances on the trunk or branch of pine tree, *Pinus densiflora* and *P. Thunbergii*, caused by a pathogenic fungus, *Cronartium quercum* Miyabe¹⁾, and it has been known in Japan as "Pine honey" since old times. S. Hattori and co-workers^{2,3)} have recently found that the sap was composed

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 * (助成研究, 課題番号 10758, 昭和 29 年度)

mainly of fructose mixed with some glucose. As yeasts, in nature, live in juice of fruit^{4,5,6,7)} or nectary, the author attempted to isolate yeasts from pine honey. This resulted in the frequent isolation of a special strain of yeast, namely *Torulopsis candida*. According to the paper of K. Saito⁸⁾, this strain ferments D-glucose and D-fructose, but not saccharose. Regarding these points, the author tested further and confirmed the facts formerly reported.

The yeast grows most favourable at 20°C. This is lower than the temperature considered favourable for many other yeasts. Relating with the two facts, behaviors towards sugars and temperature optimum for growth of *Torulopsis candida*, to the high frequency of the yeast in pine honey, one may understand that pine honey, which is the habitat of *T. candida*, is composed of only two kinds of sugar, fructose and glucose, and that the low temperature optimum for growth of *T. candida* corresponds to the fact that pine honey exudes during the winter months.

Experiments and Results

1. The first isolation; Date; Feb., 1954.

Locality and Sources; Umaki district 8 km north-east of Hiroshima city. One material (Source A) was pine honey from a protuberance of the exposed root trunk of *Pinus densiflora* from the soil (Fig. A), and the other (Source B) was pine honey from a protuberance of a branch of *Pinus thunbergii* (Fig. B). For isolation a wort medium of 10° Balling, pH adjusted to 4.8 with tartaric acid and the same containing an additional 35% glucose were both employed. The material was smeared directly on wort agar plate and this was incubated at 25°C for 4 or 5 days. All types of yeast which occurred on these plates were sorted. On the other hand a material about the size of a rice grain inserted into a test tube containing a medium, which consisted of 5 ml wort. After liquid cultures were kept at 25°C for 7 or 8

Table 1. Isolation of *Torulopsis candida* from Pine Honey (1)

Source		A [†]				B [†]	
Isolation days		February, 1954					
		14 th.		28 th.		28 th.	
Medium		solid	liquid	solid	liquid	solid	liquid
	wort	$\frac{3}{3}$ ††	$\frac{1}{2}$	$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{3}$	$\frac{1}{2}$
	wort containing 35% glucose	$\frac{2}{3}$	$\frac{1}{2}$	$\frac{2}{2}$	$\frac{1}{2}$	$\frac{0}{3}$	$\frac{0}{2}$

† Sources A & B, in text

†† Denominator of the fraction represents the number of times of attempt, numerator representing that of positive isolation

Table 2. Isolation of *Torulopsis candida* from Pine Honey (2)

Source	A†			B		C	D	E	F	G	H	I	J	K	L	
Height from ground level to protuberance where honey exudes (cm)	70			200		500	250	250	200	350	150	150	60	70	50	
Isolation days, 1955	Jan.			Jan.		Jan.	Jan.	Jan.	Feb.	Feb.	Feb.	Feb.	Feb.	Feb.	Mar.	
	16	23	30	16	23	30	30	30	6	6	6	6	6	6	27	19
Quantity of pine honey smeared	++			+		+	+	+	++	+	+	+	+	+	+	±
	3	3	3	2	1	0	2	0	3	2	2	2	2	2	2	1
Result ‡	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

† A, F, G, H, I, J, K, and L; *Pinus densiflora*, pine honey

B, C, D, and E; *Pinus thunbergii*, pine honey

± ± ±, full size of rice grain or more

±, half size of rice grain

±, trace, due to scarcity of pine honey exuded

‡ See the remarks in Table 1

days, the sediment in the test tube was streaked on the wort agar plate.

The cultures of *Torulopsis candida* obtained in the first isolation are shown in Table 1.

2. The second isolation: Date; Jan. to Mar., 1955.

Localities and Sources; 1) Umaki, Sources A and B, used in the first isolation and other Sources C, D, and E.

2) Nabara district, about 20 km north of Hiroshima city, using Sources F, G, H, I, J, K, and L (Source L is shown in Fig. C).

Honey agar* was employed as isolation medium. Cultural plates which were smeared with pine honey were kept at room temp. 8°~15°C and only the strain of *T. candida* was picked up. Table 2 shows the cultures of the present strain obtained in the second isolation. Successful isolations seemed to depend much on the quantity of pine honey smeared on the cultural plate. Successful results can be expected, when a large quantity is smeared, even to a size of rice grain.

It seems advisable to employ honey agar for isolation of *T. candida* strain for the following reasons: (1) most of the obstructive fungi are checked in this medium during the first 3 to 4 days, (2) the object strain can be anticipated merely through microscopic observation, owing to its special characteristic of forming a large oil drop within a few days in each cell of the present strain when it is placed upon honey agar.

No detailed examination was attempted to determine relations to the success of isolation according to influences in height of the sources from ground level.

Successful isolation seemed to be not related to the sorts of pine trees.

3. Identification:

Taxonomic procedures were similar to those of Lodder (1952). As saccharose is highly heat labile, this sugar was sterilized by the treatment with 70% etyl alcohol for 24 hrs. at room temp. Characteristics of the vegetative reproduction: The present strain reproduces by budding only; on wort agar, at the end of 9 days a few of multilateral budding cells were observed (Fig. G), and after 10 days primitive pseudomycelium appeared (Fig. H). Shape and size of the cells are shown in Figs E and F. Ascospore formation; absent. Pellicle formation; absent. Only glucose and fructose were fermented in both the Einhorn tube and Lindner's small fermentation test, while galactose, saccharose, maltose, and lactose were not fermented. The results of the sugar assimilation are shown in Table 3.

* Honey on market —200 gms., tap water—800 gms. The concentration of the sugar solution was 16%, measured by means of a Kyodo's hand sugar refractometer. Adding 20 gms agar, at pH 4.6.

Table 3. Sugar Assimilation of *Torulopsis candida*

Condition for culture Kinds of sugar	slant culture, 7 days, at 25°C	liquid culture, 3 weeks, at 25°C (number of yeast cells per ml)
glucose	+	624 × 10 ⁶
galactose	++	296 × 10 ⁶
fructose	++	520 × 10 ⁶
saccharose	++	288 × 10 ⁶
maltose	+	256 × 10 ⁶
lactose	±	208 × 10 ⁶
control	—	136 × 10 ⁶

++, heavy growth; +, moderate growth; ±, poor growth; —, no growth

Ethanol as sole source of carbon: weak growth. Production of starch like compounds: absent.

According to the above experimental observations, the author identified this strain as *Torulopsis candida* (Saito) Lodder.

4. Other noteworthy features:

(a) Oil drop formation:

Distinguishable globular drops taking form in the cells of the present strain a few days on wort or honey agar turned out to become a visible feature. As this drop was stained black with osmic acid and also bluish black with Regaud haematoxylin, it is evident that the drop contains lipoid. The oil drop formation were examined in liquid and solid media of wort and honey, respectively, and it was confirmed that the formation did not occur in the liquid media, but only on the solid media (Fig. D).

Table 4. The Growth of ++ *Torulopsis candida* at Various Temperatures
(10° balling wort, at pH 4.8)

Condition for culture		slant culture, wort agar			dilution plate culture on wort agar (ratios of number of colonies)			liquid culture in wort (number of yeast cells per ml)		
Expt. No.		1	2	3	1	2	3	1	2	3
	15°C	±	±	±	10	10	10	11 × 10 ⁵	8 × 10 ⁵	8 × 10 ⁵
Temp.	20°C	++	++	++	25	25	25	65 × 10 ⁵	54 × 10 ⁵	57 × 10 ⁵
	25°C	+	+	++	22	22	23	49 × 10 ⁵	30 × 10 ⁵	39 × 10 ⁵

Readings were made after 5 days

++, heavy growth; +, moderate growth; ±, poor growth

(b) The optimum temperature for growth:

The optimum temperature for growth of *T. candida* was examined at three levels; 15°C, 20°C, and 25°C, respectively. The results are shown in Table 4.

The optimum temperature for growth of this strain seems to be near 20°C.

Acknowledgment

The author desires to express his cordial gratitude and his hearty thanks to Dr. Shizuwo Hattori and Dr. Kendo Saito for their important suggestions and valuable advices.

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Explanation of Plate

Figs. A—C. Protuberances from which exudations of Sources A, B, and L in text occurred:

Fig. A, Source A, protuberance on the exposed root from soil;

Fig. B, Source B, on the branch;

Fig. C, Source L, on the trunk.

Figs. D—H, Cellular morphology of *Torulopsis candida*: (One unit scale represents 2.3 μ)

Fig. D, on honey agar, after 3 days, at 25°C;

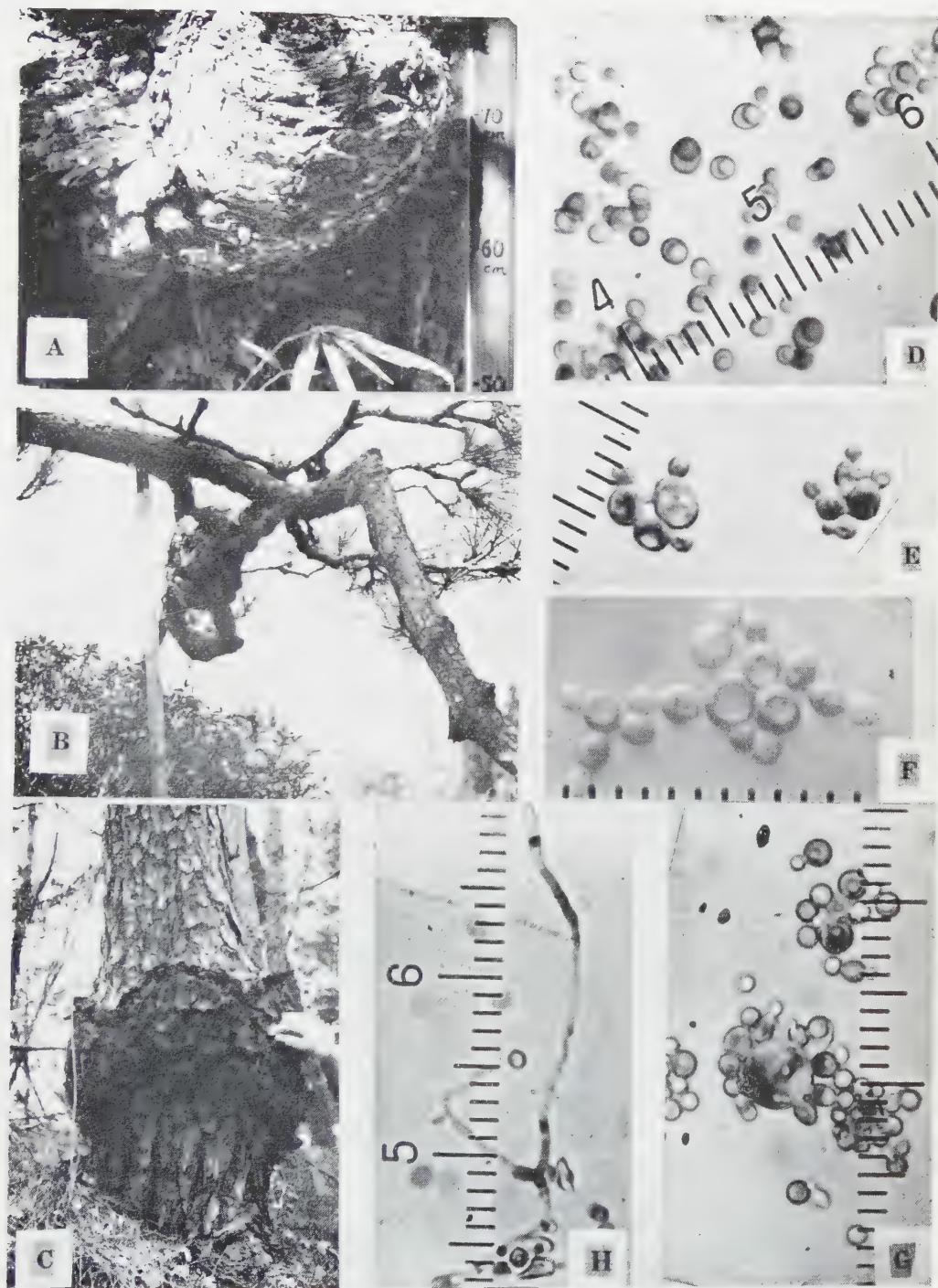
most of the cells formed oil drops.

Fig. E, on wort agar, after 3 days, at 25°C;

Fig. F, in wort, after 3 days, at 25°C;

Fig. G, giant cell budding multilaterally, on wort agar, 9 days, at 25°C;

Fig. H, pseudomycelium, on wort agar, 10 days, at 25°C.



M. YONEYAMA: Studies on Yeasts Isolated from Pine Honey

Formation of Starch in Storage Organs III

The Starch Formation in the Root-tuber of the Sweet-potato

by Shichiro HORI*

堀 七 郎： 貯蔵器官における澱粉の形成（第三報）

甘藷塊根中における澱粉の形成

Received September 9, 1955

It has been shown by the works of Hanes (1940) with potato and of Nakamura and his co-workers (1951) with a number of different plants that most of their starch storage organs contain phosphorylase. But no histochemical studies have yet been available so far concerning the distribution and activity of this enzyme in storage organs, presumably on account chiefly of the difficulty of the removal of storage starch from these organs.

In view of these situations, the writer attempted to remove the storage starch from the storage organs of some plants and succeeded in investigating phosphorylase histochemically. Previous studies (Hori, 1954) with potato tuber and maize kernel revealed that phosphorylase was generally detectable in all the tissues which are rich in storage starch such as cortex, medulla, endosperm, and embryo and also in the tissues with no storage starch such as cambium, sieve tubes, and nucellus; starch grains were generally formed in the leucoplasts in the cells of storage organs through the supply of sugars.

The present paper deals with a study of the starch formation in the root-tuber of the sweet-potato, employing the same procedure as with potato tuber and maize kernel. The results obtained differed in several important respects from those with potato and maize.

Materials and Methods

(1) Preparation of the Material

In order to investigate phosphorylase histochemically, the experimental materials had to be made free of storage starch. But the removal of the starch from the

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The writer wishes to express his sincere thanks to Professor T. Miwa of the Tokyo University of Education for his constant criticism and advice throughout the course of this study, which was carried out in the Botanical Institute of that University.

thick root-tuber of more than 7 mm in diameter was found to be very difficult. Therefore, the materials to be employed in this experiment were necessarily limited to very small root-tubers of 5-7 mm. in diameter. After the plants with roots, stems, and leaves were taken out of the soil they were washed with water free of soil particles and kept in the dark with the roots and root-tubers immersed in water. In this case, it was found favourable for the removal of the starch to leave only 1 or 2 root-tubers on each stem and cut away the rest of them from the stem. During the course of this treatment all the primary leaves fell away after about 10 days, when new buds began to grow at the leaf-axils and new etiolated stem and leaves appear at the stem tip. In this way, after about a month or more starch in the root-tuber could be removed almost entirely at room temperature of 15-20°C, but it took about two months at 10-15°C, and the complete removal of the starch was found almost impossible below 10°C. In the potato plant it was indispensable for the removal of starch to keep the tuber in air, while in the sweet-potato the disappearance of starch in the root-tuber seemed to be less affected by whether the root-tuber was kept floating on or immersed in water probably due to the absence of lenticels on the surface of the latter root-tuber as differed from the case in the former.

The disappearance of the storage starch starts in xylem parenchyma* and ends in cortex (Figs. 1, 2).

(2) Experimental Methods

For detecting the distribution of phosphorylase, hand-sections from the starch-free root-tuber were incubated in a 0.5 per cent buffered medium of Cori-ester for 24 hours and then inspected by the same method as employed in potato tuber (Hori, 1954).

For examining the starch formation from sugars, the blocks of 1-2 cm in length cut from the starch-free root-tuber were placed in various kinds of sugar solutions with one cut ends exposed to air. After keeping them in this manner for 24 or 48 hours, sections from the root-tuber blocks were inspected by the same method as in potato tuber.

Results

1. Distribution of Phosphorylase

The products formed in the cells of the root-tuber by the incubation with Cori-ester for 24 hours were very small grains stainable violet blue with iodine, bearing a striking resemblance to the natural starch grains in form and iodine colour but not in size. They appeared in the cells mainly close to the cell wall, where numerous leucoplasts had been observed when the cells were free of starch, but not in

* Xylem parenchyma of the root-tuber of the sweet-potato is the tissue portion occupying the most part of the inside of the ordinary cambium, excepting vascular bundles. In this tissue, most of the starch is stored, so that it is commonly called the "starch tissue".

the center of the cell (Fig. 5). The grains were found almost uniformly distributed in all the tissues where storage starch was to be formed, such as xylem parenchyma, phloem, and cortex, but not at all in the tissues lacking the storage starch, such as epidermis, external cortical cells, and vessels. As an exception a small amount of starchy products were formed in the cambium where no starch is stored.

2. Starch Formation from Sugars

When the block of the starch-free root-tuber was immersed in various kinds of sugar solutions with its one end exposed to air for 24 hours at the temperature of 15-20°C, starch grains were formed in cortical cells and in xylem parenchyma from certain kinds of sugars, but none from the other. It is interesting to note that the tissues kept in the sugar solutions which are incapable of starch formation showed strong plasmolysis after 24 hours of incubation, while no such phenomenon was observed in the sugar solutions where abundant formation of starch had taken place. Those sugars which caused slight plasmolysis after 48 hours of incubation formed starch but little during 24 hours of incubation.

Below the temperature of 10°C, no starch was formed at all from any kind of the sugars tested.

The formation of starch and the occurrence of plasmolysis in various kinds of sugar solutions are indicated in Table 1.

Table 1. Formation of starch and the appearance of plasmolysis in the root-tuber cells of sweet-potato in various kinds of sugar solutions

Sugars supplied	Incubation time, 24 hours		Incubation time, 48 hours	
	Starch formation	Plasmolysis	Starch formation	Plasmolys's
Sucrose	++	×	++	×
Maltose	+	×	+	○
Glucose	+	×	+	○
Fructose	+	×	+	○
Galactose	+	×	+	○
Mannose	—	◎	—	◎
Xylose	—	◎	—	◎

Concentration of sugar, 10%; Temperature, 15-20°C; Starch formation: —, none; +, trace; ++, abundant; Degree of plasmolys's: ×, no plasmolysis; ○, slight plasmolysis; ◎, strong plasmolysis.

As may be seen in Table 1, starch was formed from sucrose, maltose, glucose, fructose, and galactose. Among them sucrose was found the most favourable, and caused no plasmolysis. In this case, however, the amount of starch did not increase by further incubation than 24 hours. From mannose and xylose no starch was formed.

The starch grains thus formed showed the same appearance as the natural

storage starch in iodine colour and shape, but varied markedly in size. They were formed chiefly close to the cell wall in the same manner as the products from *Cori-ester*, but almost absent around the nucleus, where leucoplasts occurred rarely. In this respect the mode of starch formation of sweet-potato differs from that of potato tuber and maize kernel (Fig. 6).

Discussion

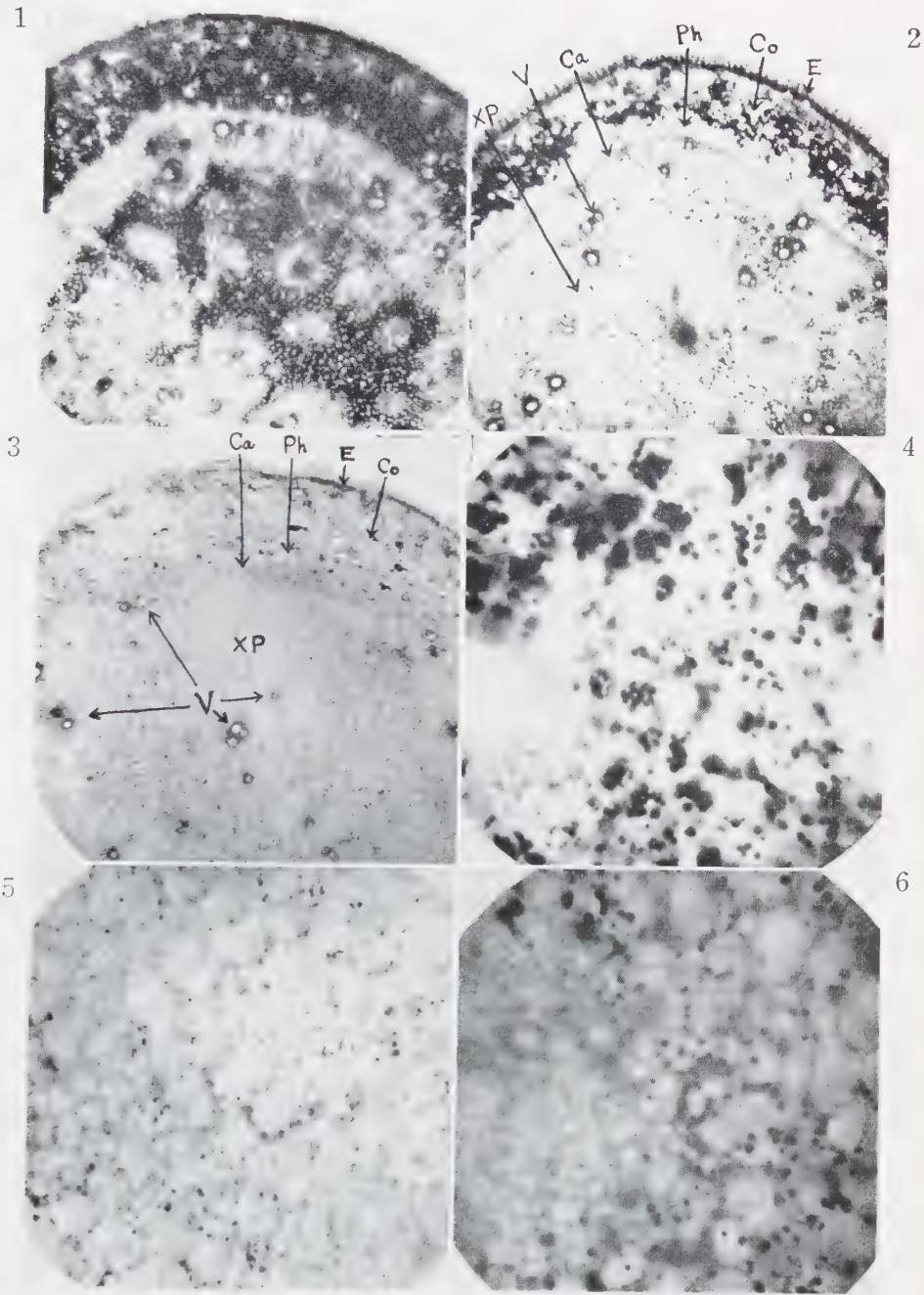
The starchy substances produced from *Cori-ester* in the cells of sweet-potato were localized almost exclusively close to the cell wall, where the presence of leucoplasts had been observed when the cells were free of starch. This observation might indicate that in the sweet-potato the products arise solely from the leucoplasts and that no phosphorylase is contained outside of the leucoplasts. In this respect the sweet-potato differs from potato tuber and maize kernel, where the starchy products appeared not only in the leucoplasts but also outside of them. Accordingly, it appears rather likely that the localization of the phosphorylase in the cell differs with the species of plants, not being necessarily limited to the plastid.

The difference in the starch formation between sweet-potato and potato or maize kernel manifests itself also in the shape and iodine coloration of the products from *Cori-ester*. In sweet-potato the products are very small grains stained violet blue with iodine, while in potato tuber and maize kernel they appeared in minute granules or branched chains of granules showing iodine colours of pure blue in the former and violet in the latter.

The noteworthy results obtained in this experiment are that there seems some relation to exist between starch formation and plasmolysis. The most favourable sugar for the starch formation was sucrose, which caused no plasmolysis of the cells of the root-tuber when the tissue was immersed in this sugar solution for 48 hours. In the solutions of mannose and xylose where no starch formation occurred at all, strong plasmolysis was observed. Maltose, glucose, fructose, and galactose all of which were available for the starch formation to a lesser extent caused slight plasmolysis when the incubation period was longer than 24 hours. The amount of starch formed from these sugar was small as compared with from sucrose. Thus it appears probable that the starch formation may be intimately related to the permeability of the sugars to the cell of the root-tuber. The failure of mannose and xylose for starch formation might be due to their low permeability rather than the inability of the cell for the conversion of these sugars to starch.

Summary

- 1) The distribution of phosphorylase and the starch formation from sugars in the root-tuber of the sweet-potato were histochemically investigated.
- 2) In the root-tuber of the sweet-potato phosphorylase was detected in all the tissues where storage starch was to be formed, such as cortex, phloem, and xylem



parenchyma but it could not be demonstrated in the tissues devoid of the storage starch, such as periderm, external cortical cells and vessels, with the exception of cambium where no starch was stored.

The products from Cori-ester were very small grains, resembling natural starch grains in shape and iodine coloration. They were formed solely in the cells with leucoplasts.

3) By the supply of various sugars through the cut end of the starch-free root-tuber, starch grains were formed in the cells of cortex and xylem parenchyma.

Of the sugars tested, sucrose, maltose, glucose, fructose and galactose were found to cause the starch formation, while mannose and xylose were ineffective.

Explanation of Plate

Fig. 1. Transverse section of the root-tuber of sweet-potato, which was kept wet in the dark for 10 days at 15-20°C, showing the storage starch beginning to disappear in the xylem-parenchymatous cells bordering the vascular bundles; stained with iodine. $\times 15$.

Fig. 2. Transverse section of the root-tuber of sweet-potato, which was kept wet in the dark for 20 days at 15-20°C, showing the storage starch remaining in the cortex; stained with iodine. E, epidermis; Co, cortex; Ph, phloem; Ca, cambium; XP, xylem parenchyma; V, vascular bundle. $\times 15$.

Fig. 3. Transverse section of the root-tuber which was entirely freed of storage starch, showing all the cells emptied of starch; stained with iodine.

E, epidermis; Co, cortex; Ph, phloem; Ca, cambium; XP, xylem parenchyma; V, vascular bundle. $\times 15$.

Fig. 4. Transverse section of the root-tuber, showing natural starch grains stored in the xylem-parenchymatous cells; stained with iodine. $\times 200$.

Fig. 5. Transverse section of the starch-free root-tuber incubated with Cori-ester solution for 24 hours, showing the appearance of starchy substance in the xylem-parenchymatous cells; stained with iodine. $\times 200$.

Fig. 6. Transverse section of the starch-free root-tuber immersed in 10 per cent sucrose solution for 24 hours, showing the starch grains formed from the sugar in the xylem-parenchymatous cells; stained with iodine. $\times 200$.

Type を異にする トウモロコシ 種子の多糖類 生成に関する酵素学的研究 II

Non-waxy トウモロコシの Q-酵素

田 中 国 治*

Kuniji TANAKA: Enzymatic Studies on the Mechanism of Polysaccharide
Formation in Maize-Seed II

Q-Enzyme of Non-Waxy Seeds

1955 年 8 月 2 日受付

著者は先に¹⁾ 糯質トウモロコシの胚乳形成にあ
ずかる wx 遺伝子の作用の本質を明らかにしよ
うとする目的から、糯質(Tokuto)及び Non-
waxy の種子 (Country Gentleman × Wisconsin
No. 690) よりそれぞれデンプン形成に關与する
Branching 酵素をとり、トウモロコシ・アミロ
ース及びグルコース-1-磷酸に対する行動をしらべ、
その際 Non-waxy の酵素では糯質の酵素と異つ
て、アミロースよりアミロペクチンへの変化がヨ
ウ素呈色法では認められなかつたことを報告し
た。糯質及び Non-waxy の種子の炭水化物の本
質的相違はアミロペクチン部分にあること^{2,3)}、
P-酵素については両者の間に相違がないと見られ
る結果が得られていること⁴⁾等よりして、これら
両 type の種子の Branching 酵素についての比
較研究は、wx 遺伝子の作用の本質を理解する上
に有力な知見を与えるように思われた。

本研究では Non-waxy 酵素の枝分れ形成作用
の機構を明らかにするために、ジャガイモのアミ
ロースを基質として Branching 酵素を作用させ、
その生成物では β -アミラーゼによる加水分解の程
度が低下するか否かを検し、更に前回の研究にお
いてヨウ素呈色法ではアミロースよりアミロペク
チン形成の認められなかつた原因を追求した。又
そこに得た結果が、実験条件の相違にもとずくも

のであるか否かを確めるために、既によく研究さ
れているジャガイモの Q-酵素について同様の実
験を行つた。それらの結果について報告する。

実 験 の 部

トウモロコシ Branching 酵素の調製——ト
ウモロコシ種子は Country Gentleman (糖質)
に Wisconsin No. 690 (デンプン質) を交配させ
て得たもので、若干の点を除き、大略前回と同じ
方法で調製した。

2 日間室温で水に浸し、膨潤させた種子 10gm.
を石英砂及び 40 cc の 0.25 M クエン酸塩緩衝液
(pH 6.0) と共に乳鉢中で塵状に粉碎した後遠心
分離して液面に浮遊した物質と共に懸濁液をと
り、これに硫酸飽和溶液を加えて 1/2 飽和とし
た。混合後溶液を 26° に加温し、直ちに遠心分離
した (2000 r. p. m. 10 分間)。析出物は液面に浮
遊したのでサイフォンを用いて液のみを取りわ
け、これに更に硫酸飽和溶液を加えて 2/3 飽和と
し、混合後遠心分離して沈澱を集めた。これを 20
cc の 0.25 M クエン酸塩緩衝液に溶解し、不溶
物を遠心分離して除いた。この液に再び硫酸飽和
溶液を加えて 60% 飽和とした後、これを冷蔵庫中
(0°) に 1 昼夜乃至 2 日間保存後生じた沈澱を遠
心分離 (3000 r. p. m. 5 分間) して集めた。沈澱
を 0.25 M クエン酸塩緩衝液 (pH 6.0) を含む
1/2 飽和の氷冷した硫酸飽和溶液で 3 乃至 4 回
(毎回 3000 r. p. m. 3 乃至 5 分間) 洗滌後得た沈
澱を 7 cc 乃至 12 cc の水に溶解した。

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かくて得た酵素液は少量のアミラーゼ及びウォスファターゼを含んでいたが、pH 6.7 では大體不活性であつた。この酵素液は、pH 6.7 に調整して用いていた。

ジャガイモ Q 酵素 ジャガイモ (Q-酵素は Barker 等⁵⁾ の方法により市販のジャガイモより調製した。100 cc のジャガイモ搾汁より得た酵素を 20 cc の水に溶解した。

β-アミラーゼ—β-アミラーゼは大豆粉末より Bourne 等⁶⁾ の方法により調製した。酵素 2.5% 水溶液を水道水に対し 2 日間透析し、生じた沈澱を遠心分離して除いた後冷蔵庫に保存した。この酵素液は α-アミラーゼ及びマルターゼを含んでいない。

ジャガイモアミロースの調製—McCreedy & Hassid⁷⁾ の温水抽出法に Haworth 等⁸⁾ のチモール法を併用して調製し、純度約 93% のものをえた。

枝分れ結合の形成機構に関する実験—先ず酵素反応中に存在する分枝形成因子アミロースに酵素を作用させ、β-アミラーゼによる加水分解の程度が低下するか否かを検した。反応条件は、0.3% アミロース (150 mg, アミロースに数滴のエタノールを加えて潤おした後 0.5 N NaOH 10 cc を加えて煮沸水浴上で溶解し、0.5 N 硫酸で中和後 50 ml. とした) 4.0 ml., 0.25 M クエン酸塩緩衝液 (pH 6.7) 2.0 ml., 酵素液 1.0 ml., 反応温度 30°。一定時間酵素を作用させた後試験管を取出し、長さ 70 cm, 直径 5 mm のガラス管を附し、100° に 10 分間加熱して酵素を失活させた。冷却後 β-アミラーゼ溶液 1.0 ml. を加えて 30° で加水分解させ、その反応液 1.0 ml. をとり出して Shaffer-Somogyi 試薬 No. 60¹⁰⁾ (以下麦芽糖の定量にはこの試薬を用いた) で定量し、Branching 酵素添加直後同様の方法で測定した対照の結果と比較した。

この β-アミラーゼ消化液 1 ml. 中には 1.5 mg の基質が含まれているが、その純度 93%, 又澱粉 0.94 gm. より約 1 gm. の麦芽糖を生成するから、消化液 1.0 ml. より生成されるべき麦芽糖量は

$$1.5 \text{ mg} \times \frac{0.93}{0.94} = 1.5 \text{ mg}$$

で、従つて Branching 酵素反応中に混在するアミ

ラーゼによる生成麦芽糖量 [x] を補正したときの β-アミラーゼによる生成麦芽糖%は

$$\text{生成麦芽糖\%} = \frac{\beta\text{-アミラーゼ作用により生じた麦芽糖 mg}}{1.5 \text{ mg} - x} \times 100$$

となる。この場合 x は β-アミラーゼ添加直後の反応液 1.0 ml. の還元力と対照における同様な測定値との差を麦芽糖として求めた。

実験の結果を Fig. 1 に示した。この場合反応中生じた還元力は麦芽糖として 5% であつた程度

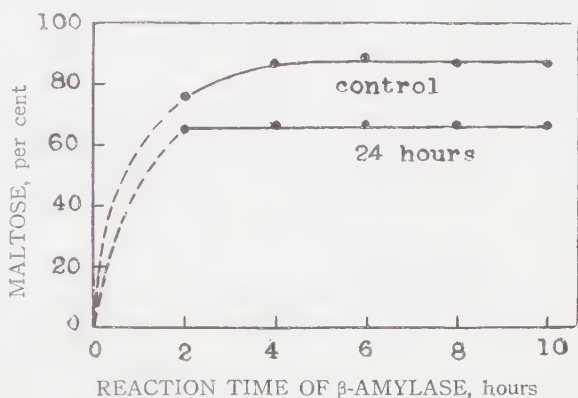


Fig. 1. Hydrolysis by β-amyase of the polysaccharide formed from potato amylose by the action of maize Q-enzyme.

少であつた。この図にみられるように、対照では 89% 麦芽糖生成がみられたのに対し、24 時間酵素を作用させたものでは約 66% にすぎなかつた。従つてこの酵素は磷酸塩の関与なしにアミロースをアミロペクチンに変化させるものであることがわかつた。

次にアミロースよりアミロペクチンへの変化に対してグルコース-1-磷酸及び無機磷酸塩を添加することにより、分枝形成が促進されるか否かを検した。この酵素標品中には P-酵素が含まれているので、新たに P-酵素を加えなかつた。この実験の結果を TABLE I に示した。

図にみられるように、グルコース-1-磷酸及び無機磷酸塩を添加した同量の β-アミラーゼによる分解限はそれぞれ 72% (P-酵素により葡萄糖-1-磷酸より合成された多糖類を補正した) 及び 68% で、添加しなかつたもの (70%) と殆んど同程度であつた。

以上の結果よりこの酵素は Q-酵素であつて、

TABLE I.

EFFECT OF GLUCOSE-1-PHOSPHATE AND INORGANIC PHOSPHATE ON THE ACTION OF MAIZE Q-ENZYME IN THE PRESENCE OF P-ENZYME Reaction temperature, 30°. Q-Enzyme action was stopped by heating after incubation period as indicated and then the reaction product was subjected to the action of β -amylase. With 1.0 ml. aliquot of the digestion mixture maltose was estimated.

Reaction mixture	Reaction time hour	Maltose produced by the action of amylase contained in the enzyme solution during the reaction time mg.	Polysaccharide produced by the action of P-enzyme during the reaction time mg.	Maltose to be formed in the reaction mixture from total polysaccharide (A) mg.	Actual production of maltose by the action of β -amylase (B) mg.	Maltose produced (B) \times 100 (A) %
A	0	0		1.5	1.33	89
	12	0.05		1.45	1.02	70
B	12	0.05	0.12*	1.58	1.14	72
C	12	0.11		1.39	0.94	68

*: calculated from milligrams of inorganic phosphate liberated from glucose-1-phosphate. The determination was made by the method of Fiske-Subbarow¹¹⁾.

- A: 0.3% Amylose 4.0 ml., 0.25M Citrate buffer (pH 6.7) 2.0 ml., Enzyme solution 1.0 ml.
B: 0.3% Amylose 4.0 ml. 0.014M Gl-1-P dissolved in 0.25M citrate buffer (pH 6.7) 2.0 ml., Enzyme solution 1.0 ml
C: 0.3% Amylose 4.0 ml., 0.25M Citrate buffer (pH 6.7) 1.5 ml., 0.2 M Phosphate buffer (pH 6.7) 0.5, Enzyme solution 1.0 ml.

ジャガイモ Q-酵素と同様にアミロースを交叉結合して枝分れを形成するものであると思われる。

枝分れ形成作用の限界に関する実験——前回の研究¹⁾においてヨウ素呈色法で検した場合、アミロースよりアミロペクチンへの変化が認められなかつたことを報告したが、これを本酵素が Q-酵素であるという事と併せて考察すると、生アミロペクチンの直鎖部分のグルコース鎖長が比較的長く、その分枝程度が低い結果ヨウ素呈色ではアミロースと著しい相違を示さなかつたことによるものであろうかと推測される。そこでこの酵素の枝分れ形成作用の限界を β -アミラーゼによる分解限についてしらべた。この実験では反応中生じた麦芽糖量 [x] は、酵素作用を測定するのと同じ組成をもつた別の反応混合液より、0.875 ml. をとり出して測定した。

実験の結果を同じ実験条件でジャガイモ Q-酵素について得た結果と比較して Fig. 2 に掲げた。この場合 Q-酵素反応中のアミラーゼによる麦芽

糖生成量はトウモロコシの場合は最終(反応時間 24 時間)で 5%, ジャガイモ酵素の場合は全然認められなかつた程僅少であつた。この図から見られるように、Q-酵素の反応の進行に伴い、 β -アミラーゼによる加水分解限は急速に低下するが、トウモロコシ酵素の場合では曲線は約 62% 附近で限界に達し、その限界はジャガイモ酵素による場合と比較して 10% 以上高かつた。又トウモロコシ Q-酵素による実験の場合には、枝分れ形成作用が限界点附近に達すると、反応生成物が沈澱してくる現象が認められた。

トウモロコシ及びジャガイモ Q-酵素によりそれぞれジャガイモアミロースより調製したアミロペクチンの性質の比較——アミロペクチン調製: 0.3 gm. ジャガイモアミロースを 0.5 cc のエタノールで潤おした後、20 cc の 0.5 N NaOH を加えて煮沸水浴上で溶かし、冷却後 0.5 N H₂SO₄ でフェノールフタレインに対し中性とし、水を加えて 100 cc とした。これに 0.25 M クエン酸塩

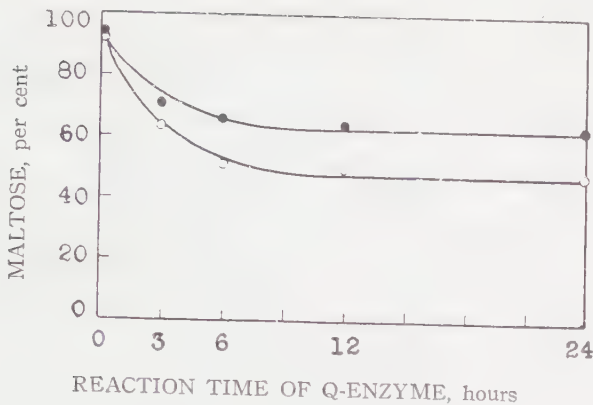


Fig. 2. Decrease of limit of hydrolysis by β -amylase of potato amylose by each action of maize Q-enzyme and potato Q-enzyme. (•) Maize Q-enzyme. (○) Potato Q-enzyme.

Reaction mixture contained 4.0 ml. of 0.3% amylose, 2.0 ml. of 0.25 M citrate buffer (pH 6.7) and 1.0 ml. of Q-enzyme solution. Reaction temperature, 30°. After heat inactivation the reaction mixture was added with β -amylase and the resulting maltose was determined.

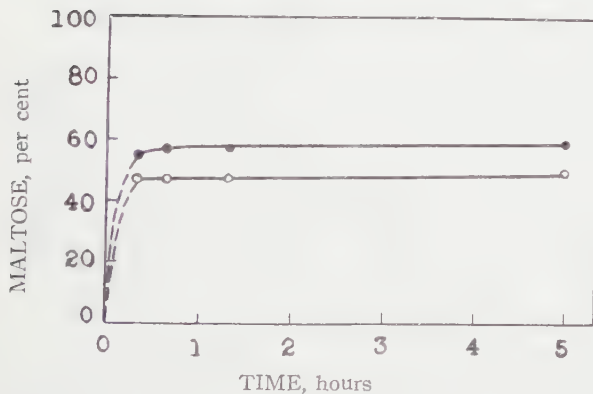


Fig. 3. β -Amylolytic of amylopectins formed from potato amylose. (•) Amylopectin obtained by the action of maize Q-enzyme. (○) Amylopectin obtained by the action of potato Q-enzyme.

緩衝液 (pH 6.7) 25 cc 及び 30 gm. の膨潤させたトウモロコシ種子より得た酵素を 25 cc の水に溶解して加えた。少量のトルエンを添加後 30° で 29 時間反応させた。反応中のアミラーゼによる

物質の分解は麦芽糖として 9% にすぎなかった。煮沸水浴中に 15 分間加熱して酵素を失活させた後、60° に冷却し、不溶物を遠沈して除いた。溶液を 1 昼夜水道水に対し、更に引き続き 1 昼夜 2 l の蒸溜水に対して透析し、エタノールを加えて 70% とした。沈澱を遠沈して集め、エタノールで 2 回、エーテルで 2 回洗滌後、 P_2O_5 上減圧乾燥した。収量約 85%。このアミロペクチンを先のアミロースと同じ仕方で溶解し、更にトウモロコシ Q-酵素を作用させたが、 β -アミラーゼによる加水分解限が低下することは認められなかった。

ジャガイモ Q-酵素によるアミロペクチンの調製も略前実験と同じ条件で行った。使用した酵素量は、ジャガイモ搾汁 100 cc より得た酵素を 25 cc の水に溶解して作用させた。72 時間反応後再び新たに調製した酵素液 25 cc を添加し、更に 70 時間反応させた。その後はトウモロコシ酵素におけると同様に操作し、約 77% の収量でアミロペクチンをとつた。

反応時間中還元力の増加は全然認められなかった。

β -アミラーゼによるアミロペクチンの分解: 0.3% アミロペクチン (溶解は先にアミロースを溶解した操作に準じた) 4.0 ml., 0.25 M クエン酸緩衝液 (pH 6.7) 2.0 ml., β -アミラーゼ溶液 2.0 ml. で 30° で分解させた場合の β -アミラーゼ分解限定結果を Fig. 3 に示した。試料中のアミロペクチンの正確な量は、アミロペクチン溶液 4.0 ml. と 7% H_2SO_4 10 cc の混合液を 100° に 3 時間加

熱し、アミロースの純度を求めた方法に準じてグルコース量を測定して求めた。図にみられるように、結果は先に得たものと略一致し、トウモロコ

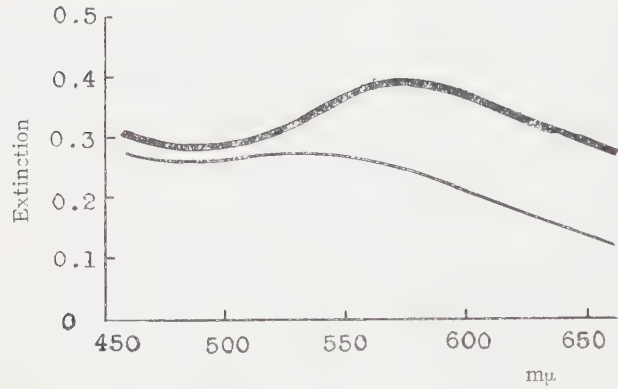


Fig. 4. Absorption spectrum of amylopectin-iodine complex of the amylopectins formed from potato amylose. (---) Amylopectin formed by the action of maize Q-enzyme. (—) Amylopectin formed by the action of potato Q-enzyme.

アミロペクチン-ヨード結合物の色調及び吸収スペクトルは、試料及び試薬混合の割合を Mc-Cready 及び Harsic⁵⁾の方法にならつて発色させ、G. E. 分光光度計で測定した。トウモロコシ酵素によるアミロペクチンの場合はヨウ素呈色は青紫色、ジャガイモ酵素による場合では赤紫色、又それらの吸収スペクトルの頂点は Fig. 4 に示したように、それぞれ 575 mμ 及び 535 mμ で、前者は後者よりも約 40 mμ 長波長のほうに片寄つていた。

両者共温水にとけ易い点は一致していたが、水溶液の安定性は異なり、トウモロコシ Q-酵素によつて調製したアミロペクチンは室温数時間で混濁を生じた。

TABLE II.
PROPERTIES OF AMYLOPECTINS FORMED FROM POTATO AMYLOSE BY THE ACTION OF MAIZE Q-ENZYME AND POTATO Q-ENZYME.

Property	Source of Q-enzyme	
	Potato tuber	Maize seed
Solubility in hot water	Easily soluble	Easily soluble
Stability in water	Stable	Unstable
Limit of hydrolysis by β-amylase	49 %	59 %
Colour of iodine complex	Reddish-purple	Bluish-purple
Maximum of absorption spectrum of iodine complex	535 mμ	575 mμ
Colour of β-dextrin-iodine complex	Red	Violet

シ及びジャガイモ Q-酵素で調製したアミロペクチンの β-アミラーゼによる分解限はそれぞれ 59 % 及び 49 % であつた。

アミロペクチンの性質の比較: β-アミラーゼによる分解限が一定した後、それぞれその 1 cc をとり、0.005 N ヨード液 2 滴を加えてヨウ素呈色をしらべると、ジャガイモ酵素によるアミロペクチンの β-デキストリンが赤色を呈したのに対し、トウモロコシ酵素によるものは堇色であつた。

これら両アミロペクチンの性質を総括して TABLE II に示した。即ち、ジャガイモ Q-酵素によつて調製したアミロペクチンの性質は、従来の研究結果とよく一致しているのに対し、トウモロコシ酵素によつて得たアミロペクチンはそれと種々の点で異なつてゐることを示している。

考 察

以上の実験結果より、ここに得られたトウモロコシ Branching 酵素が Q-酵素であることが明ら

かにされると共に、前回の研究においてアミロースよりアミロペクチンへの変化がヨウ素色の肉眼的観察では認めることができなかった点も推測され得る。即ち本酵素によって形成されたアミロペクチンはそのヨウ素結合物の光吸収の頂点が多少長波長部に片寄っているからであり、 β -アミラーゼによる加水分解限が高いことから、おそらくアミロペクチンの直鎖部分の平均グルコース鎖長が長く、その分岐は、直鎖に比べて少ないことを意味するものである。又他のトウモロコシのアミロペクチンとここに得たものとを比較してみないとはいえなかったことを述べることはできないが、 β -アミラーゼによる分解限が高いこと、及びその抽出液が比較的不安定であることは、Meyer 等¹²⁾がトウモロコシのアミロペクチンについて得た結果とよく一致している。また Larnier¹³⁾は、通常の Branching 酵素の Amylo-(1,4 \rightarrow 1,6)-transglucosidase はその作用の本質においては Q-酵素と異っているものではなく、酵素の特異性の相違にあるものとし、その性質がグリコーゲン形成に重

要な意味をもっていることを指摘した。ここに得られた事実及びこれらの知見は、Non-waxy トウモロコシ種子におけるアミロペクチンの構造を規定する上に、本酵素が重要な役割を行つていることを示唆する。

一方 Non-waxy トウモロコシの一種である糖質の下部には、少量のフィトグリコーゲンが含まれていることが知られている¹⁴⁾。Hassid¹⁵⁾等によるとこのグリコーゲンは動物性グリコーゲンに類似し、 β -アミラーゼによる分解限は 47%と報告されている¹⁶⁾。したがってここに得た実験結果は、このグリコーゲンはアミロペクチンより本酵素の作用によつて形成されるものではないであることを示唆する。

本研究において有益なご示唆を頂き、また論文のご校閲を賜つた東京教育大学三輪知雄教授に対して深謝する。又トウモロコシ材料のご提供を頂いた農林省農業技術研究所山崎義人博士及び吸収スペクトルの測定についてご便宜を頂いた大阪国立工業試験所福田保氏に対し深謝する。

Summary

The branching enzyme of non-waxy maize seeds (Country Gentleman \times Wisconsin No. 690) was found to belong to the Q-enzyme type, and it differed from potato Q-enzyme producing a slightly different amylopectin. The amylopectin produced was saccharified by soybean β -amylase to 59~62% (calculated as maltose), and gave an iodine complex with a maximum at 575 m μ in absorption spectrum, while potato Q-enzyme yielded an amylopectin with a limit of saccharification by β -amylase of 49% and with an absorption maximum of iodine complex at 535 m μ .

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タバコ属植物の細胞遺伝学的研究* VI

タバコと他の種との交雑種の減数分裂

竹 中 要**

XO TAKENAKA: Cytogenetic Studies of *Nicotiana* VI.

On Three Hybrids between *N. tabacum* and Other Species

1955 年 9 月 12 日受付

本研究の目的は (1) 種間交配による育種の優良形質の発見, (2) 雑種の減数分裂異常を利用しての優良劣悪両形質の分離, (3) タバコ属の核学的研究方法による系統進化の究明である。

材料と研究方法

材料 *N. trigonophylla* ($n=12$), *N. undulata* ($n=12$), *N. rustica* ($n=24$) は前の報告 (1951, 1953a) にて吟味したものを使った。これ等と *N. tabacum* ($n=24$) の品種との間に交配を行つた。*N. trigonophylla* \times *N. tabacum* は 1951 年に行つたが失敗した。1951 年には逆交配の *N. tabacum* \times *N. trigonophylla* を行い、種子を得た。*N. undulata* と *N. tabacum*, 及び *N. rustica* と *N. tabacum* との間には多数の正逆交配を行つたが、1951 年において得られた蒴果からは求める雑種は得られなかつた。1952 年においては *N. undulata* \times *N. tabacum* (Odaruma) と、その逆交配とをそれぞれ 8 花と 64 花について行い、前者で 1 個と後者で 2 個との蒴果を得た。そして前者からのみ少数の雑種を得た。*N. rustica* \times *N. tabacum* と、その逆交配を再び 1952 年におこなつた。*N. tabacum* (Odaruma) \times *N. rustica* (Afghanistan, Maholoca) を 128 花について行い 2 蒴果を得たし、逆交配の *N. rustica* (Afghanistan) \times *N. tabacum* (Odaruma) を 157 花について行つて 3 蒴果を得た。これ等の蒴果から得られた種子を蒔いて、1953 年において、*N. tabacum* (Odaruma) \times *N. rustica* (Afghanistan) から僅かに 1 本の求める雑種を得た。

N. trigonophylla は北メキシコ原産のやや細い植物で、花は小形で淡緑白色である。トリゴノフィラ節に入れられている。*N. undulata* はペルー原産のやや小形の植物で、花は小形、淡黄緑色を呈する。パニキュラータ節に属する。*N. rustica* は喫煙用にもなり、品種は多数ある。これは *N. paniculata* と *N. undulata* との天然交配による複二倍体と考えられるから、パニキュラータ節に入れる。植物体はタバコ程大きくはないが、一見頑強に見える。花はやや大きく黄緑色を呈する。

上に述べた組合せの交配、すなわち *N. tabacum* \times *N. trigonophylla*, *N. undulata* \times *N. tabacum*, *N. tabacum* \times *N. rustica* の F_1 の花粉母細胞の観察用プレパラートの製作は鉄醋酸カーミンによるなすりつけ法を主とした。しかし時にはカルノア氏液にて蒴を固定した後、塩酸にて加水分解し、醋酸オルセインによる押しつぶし法を併用した。

観 察

(1) *N. tabacum* ($n=24$) \times *N. trigonophylla* の F_1
草型は母の *N. tabacum* よりやや小さく、葉形は両親の中間を示す。花色は淡紅色で *N. tabacum* に近いが、幾分 *N. trigonophylla* に似て淡い。やや雑種強勢を示す。種子はつけない。

減数分裂 第一分裂: 減糸期、複糸期において染色糸は接着してネジレを示すことはなく、ただ数本のものに端部接着が見られるだけである。移動期においても介在キアズマ、両端キアズマは見られず、数個のものに一端キアズマ (接着か) が見られるだけである。

第一中期においては一価、二価稀れには多価染色糸が見られるが、一価染色糸の多数は核板を構成することなく、早期に機会的に両極に分配される。核板は二価と多価染色糸のほか少数の一価染

* Contributions from the National Institute of Genetics, Japan, No. 123.

** National Institute of Genetics, Misima, Japan.
(国立遺伝学研究所)

色体によつて構成される。二価と多価染色体との数は 0~11 で、5 と 6 が最も多い。多価染色体は主に三価である。



Fig. 1. F₁ *N. tabacum* × *N. trigonophylla*, IM, side view, 7II+22I

第 1 表 *N. tabacum* × *N. trigonophylla* F₁ の第一中期核板構成

二価と多価染色体数	0	1	2	3	4	5	6	7	8	9	10	11	12	合計
観察数	1	4	8	15	12	28	28	14	13	6	0	1	0	130

第一中期の核板を主とする材料においては太糸期〜第二次期のものもつと見られるが、また三分子期や二分分裂期の主とするものにおいても第一中期のものが相当数存在する。これらの場合、第一中期核板の二価染色体の数に後者は前者のものより少ない。

第一後期になると二価染色体は両極に分れるが、多価染色体の構成分子と一価染色体とは機会的に両極に分れる。染色体橋は二価と多価染色体に由来するものが多いが、稀には一価染色体に由来のものもある。第一中期において核が一価染色体で、均等分裂をする母細胞が稀に見られる。

第二分裂：第二分裂中期においては大多数の染色体は均等分裂をするが、稀には均等分裂しないで早期に核板を離れて極に向うものがある。第二分裂後期にも染色体橋が相当数見られるが、その中には第一分裂の染色体橋の残存物もある。第二分裂中期、後期において、稀に第二分裂を中止するため、母細胞が二分子のままで花粉粒生成に入るもの、また 1 娘核は二分するが、他の 1 娘核は二分しないで、三分子で花粉粒生成に入るものがある。第一と第二の核分裂を同じて核外に放出される染色体が少数存在する。

四分子期：以上のような分裂過程であるから多胞子形成によつて微細胞子をもつものが相当数に達するし、二分子、三分子形成による大形胞子も少数見られる。

(2) *N. undulata* ($n=12$) × *N. tabacum* ($n=24$) の F₁

外形は全体として *N. tabacum* により多く似ていて、幹の大きさも *N. tabacum* と殆んど同大である。葉も *N. undulata* より大きいが、葉形は幾分 *N. undulata* に似た点がある。花色は黄緑を帯びた紅色である。雑種強勢を示す。種子はつけない。

減数分裂 第一分裂：前期の太糸期の頃迄は正常の植物の染色体行動と目立つた差異はない。移動期においては正しい両端キアズマ、介在キアズマは見られないが、大小様々の染色体が一端をもつて軽く接着するものが見られる。時に端部接着の多価、環状多価染色体が存在するし、また非端部接着の染色体も見られる。

第一中期に於いては多数の一価と少数の二価、稀に一価染色体が見られる。二価及び三価の染色体には密に接合するものは少なく、多くは疎に接合する。また二次対合と見るべき二連及び三連、又はそれ以上の多連の染色体が存在する。第一中期における染色体の内一価のものの多数は早く両極に行くが、若干数の一価染色体と二価及び三価染色体の全部とが核板を構成する。第二表は第一分裂中期核板の二価（三価も含めて）染色体の出現頻度である。この表において両極に対し縦列せる二価及び三価染色体のみが掲げられており、横列せる接着型の二連乃至多連型は二次対合と見なして除外した。しかし二次対合も部分相同に基づくものがあることは、移動期における染色体の接着数が、第一中期の接合数より多いこと、及び移動期に赤道板上の染色体の動くことから推察される。この表で見られる如く 4 個の二価染色体のものが最大多数である。

第 2 表 *N. undulata* × *N. tabacum* F₁ の第一中期核板構成

二価と多価染色体数	0	1	2	3	4	5	6	7	8	合計
観察数	1	4	13	27	34	15	13	5	1	113

第一後期においては染色体橋が見られる外に、両極外に放出される染色体も存在する。但しその数及び頻度は低い。そして核外に放出されるものは甚だしい。

第二分裂：第二分裂においては姉妹染色体が同



Fig. 2. *F₁ N. undulata* × *N. tabacum*. a, early diaphase, 3III+9II+9I. b, IM, side view, 2II+30I+1 jointed chromosome, the jointed chromosome consisting of two chromosomes: c, tetrad stage, abnormal nuclei and fragmental chromosomes.

時的に両極に分れるものの外に早期に分れるもの遅れて離れるものなどが相当数見られる外、染色体橋も他の雑種よりやや多く見られる。しかし第二分裂終期において核外に放出されている染色体数は少ない。そのためか核内で染色体の集団から離れている染色体が相当数ある。その結果として四分子期に4小孢子以外の微細胞子をもつ多胞子形成の頻度は低いが、小胞子内に微細核を含むものは割合多い。

(3) *N. tabacum* ($n=24$) × *N. rustica* ($n=24$) の F_1

外形は一見 *N. tabacum* に似ているが、詳しく見ると *N. rustica* の形質もまじつて現れている。葉は大きく厚く、花色は黄色を底に含んだ淡紅色である。雑種強勢を示す。種子は稔らない。

減数分裂 第一分裂: この雑種には *N. tabacum* と *N. rustica* とからそれぞれ24箇の染色体がきているわけであるから、全染色体数は48箇である筈である。しかし減数分裂第一中期では、染色体数の多いためと不規則行動のために、完全に算定することは困難であつたが、僅かに数個の美しい像で、全数で48染色体であることを確めた。

第一中期核板は数箇の二価染色体と多数の一価染色体とからなっており、往々二箇以上の多価染色体も見られる。第3表はこの核板構成状態である。

第3表 *N. tabacum* × *N. rustica* F_1 の第一中期核板構成

二価と多価染色体数	0	1	2	3	4	5	6	7	8	9	10	合計
観察数	0	4	6	17	33	13	11	7	1	2	1	95

表で見られる様に4箇の二価染色体が最大多数

である。この第一中期における一価染色体は、既に相当数両極に先行している、外核板内にも核板附近にも存在する。これ等の数の分布は不規則である。また二次対合が相当数の染色体の間に存在し、その数は不定である。しかもその二次対合は二次接着とも申すべき密着型が多い。この接着染色体を構成している染色体の数も形も不定である。核板で接合している二価染色体にも、相互間に大きさの不同が見られる。

第一後期において二価染色体は両極に分配されるが、一価及び多価染色体の分配は不定である。またしばしば染色体橋が見られる外、多数の染色体は紡錘体外に放出される。

第二分裂: 第二分裂中期の核板には姉妹染色体の対合以外に、第一分裂時の多価染色体の、或は二次対合(接着)の何れの残存か決定できないが、端と端とで接着した多連染色体がしばしば見られる。これ等は姉妹染色体のように正しくは両極に分れない。少数のものは早期に極に向うし、染色体橋を作るし、或は主紡錘体外に放出される。

四分子期: 第一、第二分裂が不規則であるにかかわらず、多胞子現象は余り著しくない。

論 議

N. tabacum × *N. trigonophylla* に成功した人は、筆者の取調べた範囲にはない。ただ Christoff (1928) がこれの逆交配で不完全な実生を得ただけである。しかし *N. tabacum* の一方の先祖をなすと思われる *N. tomentosa* 系のものと、*N. trigonophylla* との間の交雑には成功した人々がある。Goodspeed (1934) は *N. trigonophylla* × *N. tomentosiformis* と *N. trigonophylla* × *N. tomentosa* に成功して、前者では0~9の、後者では2~11の二価染色体を、第一分裂中期で見ている。それに対して Kestoff (1941-43) は *N. trigonophylla* × *N. tomentosiformis* で0~8の二価染色体を見ているが、その内で2~5が多く、また *N. trigonophylla* × *N. tomentosa* では2~10の二価染色体が多いことを報告している。そして両者ともに稀に僅少の一価染色体を示すことを観察した、しかしこれ等の雑種 F_1 は自家授粉でも他種との交配でも種子をつくらなかつた。



Fig. 3. F_1 *N. tabacum* \times *N. rustica*. a, IM, polar view, $4II+20I+8$ jointed chromosomes, of which jointed chromosomes are composed of two or three chromosomes. b, IIM, polar and side views, showing some undivided chromosomes and chromosome fragments.

このように *N. trigonophylla* は *Tomentosa* group との間に雑種をつくるのであるから、*N. tabacum* との間に雑種を生じ得ることは考えられる。*N. tabacum* \times *N. trigonophylla* の F_1 の第一中期において、観察は0~11の二価染色体を観察したが、そのうちで5~6が多数であった。すなわち *N. tabacum* と *N. trigonophylla* との染色体の親和性は、*N. trigonophylla* と *Tomentosa* group との親和性と余り大差がない。従つて *N. tabacum* の2の subgenome の内 *Sylvestris*-genome は *N. trigonophylla* の genome との間には親和性は殆どないものと推察される。

N. undulata \times *N. tabacum* とその逆交配の成績も筆者の調べ得た範囲の文献には載っていない。また *N. sylvestris* 及び *Tomentosa* 系のもとの *N. undulata* との間で交配にも成功した記録がない。しかるに *N. undulata* \times *N. tabacum* の F_1 第一中期においては0~8の二価染色体が見られ、殊に2~5のものが多いため、そしてその数は *N. tabacum* の2の subgenome 間の染色体接合数よりも僅かに多いから、*N. undulata* の genome と *N. tabacum* の genome との間には僅少ではあるが、親和があるものと考えられる。

N. tabacum \times *N. rustica* には Eghis (1927) が成功し、Rybin (1927) がその細胞学的研究を発表した。また Eghis (1933) はこの逆交配にも成功した。しかし East and Hayes (1912), Savelli (1927), Christoff (1928), Kostoff (1930, 1937) 及び Ternovsky (1935) はこの逆交配にのみ成功した。*N. tabacum* \times *N. rustica* F_1 の外形は Kostoff (1941-43) の記載した *N. rustica* \times *N. tabacum* F_1 とよく一致する。Kostoff は彼のつくつた雑種で5~24の二価染色体を見たが、Christoff (1928) と Ternovsky (1935) はごく少数の二価染色体を観察したにすぎなかつた。筆者は *N. tabacum* \times *N. rustica* F_1 において1~10の二価染色体を観察したが、3~6が多数で、4が最大多数であつた。しかも既に *N. tabacum* の2の subgenome, *sylvestris* と *tomentosa* との間にも部分相合の染色体のあることを報告した(竹中, 1936b), また *N. rustica* の2の subgenome, *paniculata* と *undulata* との間にも部分相合の染色体が若干ある(未発表)から、上に述べた親和(接合)数が総て *tabacum* genome と *rustica* genome との間の相合または部分相合の染色体数であるとは断定できない。しかし稀れに三価以上の染色体のあること、多数の二次接合のあること、及び *N. tabacum* \times *N. paniculata* F_1 における染色体視度度が、*tabacum* の2の subgenome 間の親和度よりも高いことから、*tabacum* genome と *rustica* genome との間には少数の部分相同染色体があるものと推察される。

Kostoff (1941-43) は *N. rustica* \times *N. tabacum* F_1 の自家授粉では種子を得なかつたが、親と雑種との交配では種子を得た。筆者もまた *N. tabacum* \times *N. rustica* F_1 の105箇の花の自家授粉では種子を得なかつたが、この雑種を母として *N. tabacum* を父とした場合には72箇の花で2箇の着果を見た。

Abstract

The three species, *N. trigonophylla*, *N. undulata* and *N. rustica* were crossed reciprocally with *N. tabacum*. The crosses *N. tabacum* \times *N. trigonophylla*, *N. undulata* \times *N. tabacum* and *N. tabacum* (Odaruma) \times *N. rustica* (Afghanistan) gave some seeds, while no seeds were obtained from the three remaining crosses. No

report on the hybrids *N. tabacum* × *N. trigonophylla* and *N. undulata* × *N. tabacum* or their reciprocal crosses has been published, so far as I know.

In external characters the F_1 *tabacum-trigonophylla*, is somewhat smaller than *N. tabacum*, the leaf form is intermediate between those in the parents, and the flower colour is a pale red. F_1 *undulata-tabacum* is more similar to *N. tabacum* than to *N. undulata*, but its flowers are pale yellowish red, showing an intermediate color between the two parents. The morphology of F_1 *tabacum-rustica* agrees with that of Kostoff's description of the reciprocal hybrid (*N. rustica* × *N. tabacum*). These three hybrids were all vigorous.

All the hybrids mentioned above, showed considerable irregularities in the meiotic behaviour of the PMC's. Polysporous PMC's were often observed, and the hybrids were completely sterile.

At first metaphase in F_1 *tabacum-trigonophylla*, 0-11 bivalents, mostly 5-6, were counted. In F_1 *trigonophylla-tomentosa* and F_1 *trigonophylla-tomentosiformis*, Kostoff (1941-43) observed 2-10 and 0-8 bivalents, respectively. Accordingly, the chromosome conjugation in F_1 *tabacum-trigonophylla* is assumed to be caused mostly by semi-homologous chromosomes between the *trigonophylla* genome and the *tomentosa* subgenome, not the *sylvestris* subgenome, of *N. tabacum*. In F_1 *undulata-tabacum*, 0-8 bivalents, mostly 3-5, were observed. In this hybrid the number of the bivalents is a little more than that of the chromosome conjugation in haploid *tabacum* or F_1 *sylvestris-tomentosiformis*. Accordingly it is assumed that a few semihomologous chromosomes are present between the *undulata* genome and the two subgenomes of *N. tabacum*. In F_1 *tabacum-rustica*, 1-10 bivalents (also multivalents) were formed at the first metaphase and some secondary associations were observed at first and second metaphases. Christoff (1928) and Trenovsky (1935) also observed a small number of bivalents in F_1 *rustica-tabacum*, while Kostoff (1941-43) found many bivalents, as many as 5-24, in the same hybrid. The cause of these different results was not determined.

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ササゲの子葉における不定根の形成

埜

順*

Jun HANAWA: Formation of Adventitious Roots on the Isolated
Cotyledons of *Vigna sinensis*

1955 年 9 月 19 日受付

高等植物における再生についての研究は、従来胚生組織、組織培養又は化学的誘導に主眼を置く植物体のいろいろな部分に対する効果などに限られてなされて来た (Bloch, 1941 1952; Swingle, 1940, 1952 参照)。しかし子葉の再生現象についての研究は余り多くない。Nakano (1924) はソラマメ、ペニバナインゲンマメ、エンドウ、ダイズ及びトウモロコシの子葉の癒傷について研究し、子葉切断面におけるカルス形成には極性が見られて、基部に向つた断面には、先端に向いた断面におけるよりも多くのカルス形成があることを報告した。La Rue (1933) は 19 科 45 種の植物について子葉の再生を研究し、そのうち 41 種では分離した子葉に根を生じ、22 種では不定根が形成されることを見た。また Lee (1950) はトマトの発芽植物の各器官を分離して人工培養し、分離培養された子葉は、植物体上に附着している場合よりも、速かに大きな生長を示し、また多くの不定根を生ずることを見た。Carlson (1953) はアブラナ及びダイコンの子葉を分離して培養し、不定根の形成過程を解剖学的に観察した。

筆者はササゲ *Vigna sinensis* の子葉における不定根形成の過程とその不定根の内部構造について観察を行い、不定根の発生に関する子葉の特殊なはたらきについて考察しようと思う。

材料と方法

ササゲ *Vigna sinensis* の子葉を用いた。子葉

を培養するには次のようにした。種子をウズブルソンの 0.25% 溶液に 3 時間つけて消毒、次に滅菌蒸留水で洗つてペトリ皿に入れ、滅菌蒸留水に浸して 28°~30°C に 24 時間保つた。次で 0.1% 昇汞水で 2 分間洗つてから種皮を取除いた。そして次の三通りの仕方で子葉を胚軸から分離した。(1) 胚軸を子葉から引きはがしたまゝにしておく。この場合には子葉の基部に胚軸の組織の薄片が附着している。(2) 子葉の中肋に垂直に、胚軸への附着点から 2 mm ぐらい離れた処で滅菌した安全カミソリの刃で切り離す。子葉には胚軸のいかなる薄片も残っていない。(3) (1) 又は (2) のようにして分離した子葉を中肋に垂直な面で切断して、先端と基部とのほぼ等しい大きさの二片に分けた。

培養基には Knop 氏液に 2% 蔗糖, 0.1 g/l の乾燥酵母エキス、及び 2% 寒天を加えたものを用いた。pH 6.5 に調整し、これを 50 cc の三角フラスコに 20 cc つまみ入れ、切り取つた子葉をこの上に横たえた。これを室温散光中で培養した。

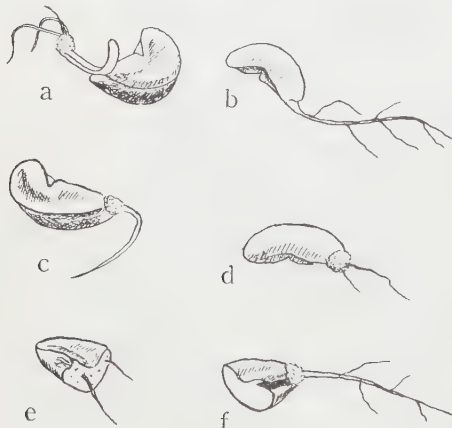
予め不定根の出現に要する時間を確めてから適当な時期に子葉又は発根しつゝあると思はれる部分を取つて、フォルマリン・酢酸・アルコール混液で固定した。これをパラフィン切片として Delafield のヘマトキシリンで染色して観察した。また発生した不定根を生長させてから、同様に固定、染色してその中心柱の構造を見た。

不定根の発現

培養基上におかれた子葉は 2~3 日たつと緑色になり吸水して重量と体積が増してくる。子葉を

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胚軸からはがしただけの場合には 2~3 日で子葉の基部について残っている胚軸の破片が伸び始め、多くの場合 1 cm 程の長さになり、4~5 日たつとその先端から発根する。胚軸が殆ど全く残っていない場合には、このように長く伸び出すことはなくて、たんに塊状のふくらみが出来てそこから根が出てくる(第 1 図 a, b; 第 2 図)。これらの場合には 7 日目ぐらいから腋芽が発達しはじめ急速に大きくなって、腋芽が出て 2 週間ぐらいたつと子葉は普通の発芽の場合と同じように全く萎縮してしまう。この培養方法の場合には胚軸からの発根と子葉そのものからの発根とを厳密に区別することが出来ない。胚軸の残片が 1 cm も長く伸び出してその先端から根を生ずる如きは明らかに子葉からの発根ではない。Lee (1950) も述べている如く、分離して培養されると、胚軸や子葉は著しく発根しやすくなる。



第 1 図

次に子葉の胚軸への附着部を完全に切り捨てた場合には、子葉からの発根であることは疑ない。

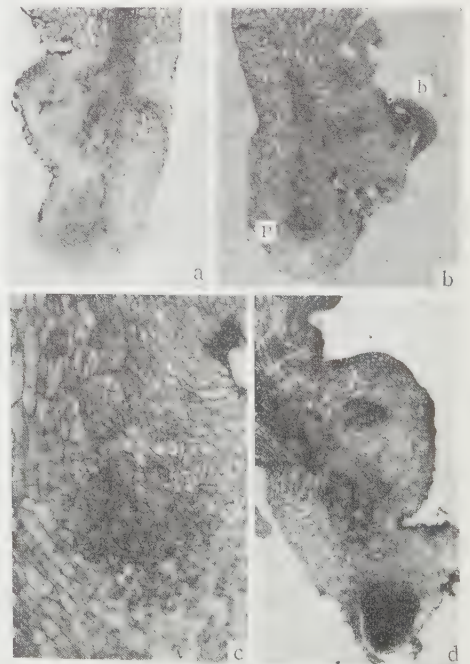
培養を始めて 3 日目ぐらいたつと、切断面に白いカルスのふくらみが発達して来て、5 日目ぐらいにそのカルスを貫いて根が現れる。太い根が一本も出ることもあり、またそれより細いのが二本以上出ることもある(第 1 図 c, d)。胚軸附着部を切り除いてあるからこの場合には決して腋芽は現れなかつた。

次に第 3 の場合、即ち子葉を基部と先端部とに二等分した場合には、基部の半分からは、もし胚軸の小片がついていれば容易に発根し腋芽もよく発達する。また胚軸附着部を切り取ってしまった

場合には、そこにカルスが出来て、それから根が出ることは第 2 の場合と同様である。先端部の半片では、まづ主葉脈の断面にカルスの小さなふくらみが出来て、5 日目ぐらいたつとそこから発根してくる。次で側脈にも同様にして発根が起る。最も多数発根した場合は、5 本の不定根が生えた(第 1 図 e, f)。この場合、基部の半片の先端に向いた切断面には決してカルスも作らず発根も起らなかった。Nakano (1924) や Carlson (1953) の観察と同じく、明かに発根の極性が見られた。

解剖学的観察

子葉に胚軸の小片が附着したまゝの培養では、この胚軸の残片が、長く伸び出すか或は不規則にふくれて来ることは前述の通りである。この塊状の組織の内部は複雑な維管束系をもち、管束の配列は全く不規則で一定の方向に走るということがない(第 2 図 a, d)。これは維管束組織という



第 2 図

よりも、盛な組織の増殖によつて生じた維管束要素のかたまりである。4 日目ぐらいまではこの組織は増大して行く。この組織の増大は、附着して残っている胚軸組織の細胞の成長と、主として維管束の柔細胞におこる細胞分裂とによるものと思

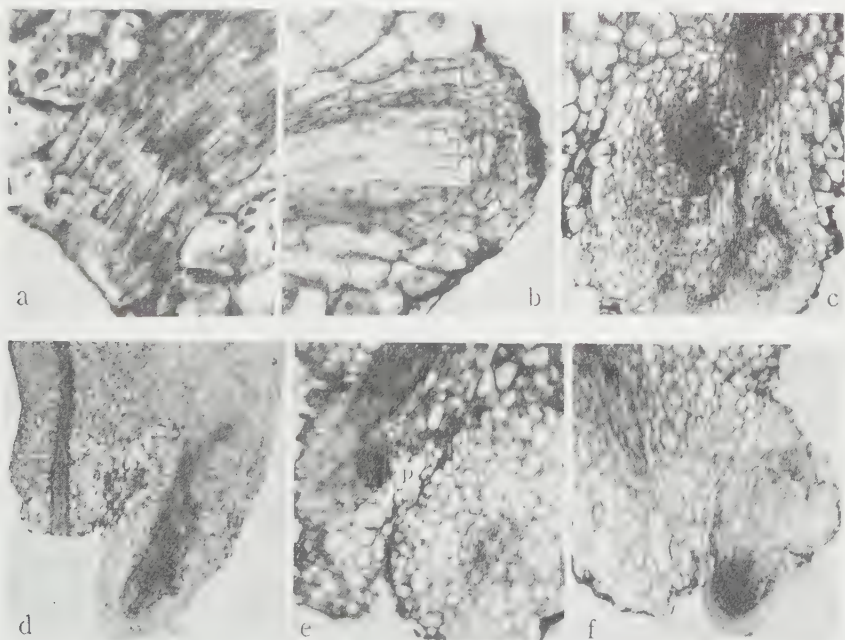
われる。4 日以後になるとこの組織の増大は次第に停止する。その頃にこの組織のかたまりの下端に厚く、大細胞の層が形成され、この層の細胞質を密に持つ一群の密集した細胞が見られる(第2図 b, c)。その位置は必ずしも一定していないが、維管束組織の末端に出来ることが知られる。これが不定根のもとである。第2図 b 及び c には根の原基である細胞群の中心、外縁に於いて分裂を起しているのが見られる。そしてその外縁の細胞層はすでにこわれかみついていて、不定根はこゝを貫いて出てくることが考えられる。根の原基はやがて明らかに根として編成され、周りの細胞層を貫いて発生してくる(第2図 d)。はじめ根の維管束と子葉の維管束との間はなされていないが、やがて胚軸のふくらみの中の不規則な維管束を通じて両者の連絡がなされるものと思われる。培養3日目以後いから、胚軸の上部には腋芽が発達し始める(第2図 b)。腋芽と不定根とが子葉との間に維管束の連絡を持つようになると、腋芽も不定根も共に急速に成長し、子葉は間もなく萎縮してしまう。

もし胚軸の残片が、塊状の組織にならずに長く伸びる場合には、胚軸の維管束は正しく上下の方向に走る。その先端に発生する不定根と胚軸及び

子葉との維管束系の連絡は前の場合よりも容易であると思われる。

第3図 a を切り出した子葉及び上下の二片に等分した子葉からの発根の過程は同じであるから、次に同時に述べる。

これらの子葉を培養して 2~3 日たつと切口が褐色になつてくる。これを組織標本にしてみると、切断面の葉肉細胞は破壊されていて、その残骸が附着して褐色を呈する。これれなかつた最外側の細胞の外側の細胞膜はクチン化を起して肥厚している(第3図 b)。葉脈の切断面には未だ変化が起つていない(第3図 a)。培養3日後になると、切断面下の数層の細胞には、それまでいつばいにつまっていた澱粉粒が全く見られなくなっている。そして葉脈の切断面には、切断面に平行な細胞分裂が起つている(第3図 b)。葉脈のはじめはつきりした木部や篩部の分化が行われておらず、そこには仮導管細胞が並んでいて、その中には夥粒がいつばいにつままっている(第3図 a)。しかし3日以後、葉脈の端に細胞増殖が起つてくると同時に、これらの夥粒は消失しその細胞は形態的にも明かに導管へ分化する(第3図 b)。葉脈の切断面に細胞分裂が起ると共に、葉脈の周りの葉肉細胞も少しばかり分裂をするので、葉脈の端



第 3 図

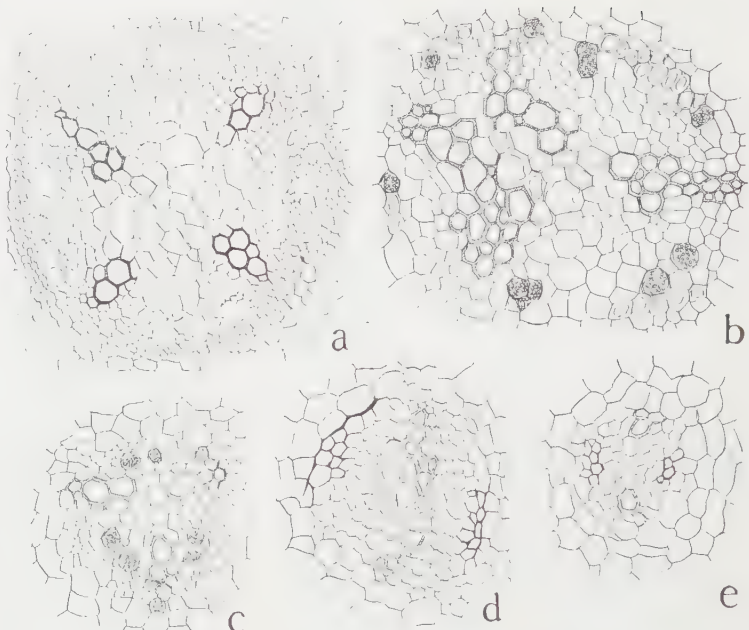
に白いカルスのふくらみが生ずる(第3図 b d)。このカルスは、子葉基部を切り捨て培養した場合に最もよく発達し、切口全部を覆う。また子葉を半分に切つた場合には、先づ主葉脈に形成され、更に数日後には細い側脈にも生ずるが、ともに余り大きくはならない。

根の原基は培養5日頃、このカルスの中に、葉脈に接してその外側に分化しはじめる。即ちカルスの中に伸びた葉脈の節部柔細胞が分裂を起して、一団の細胞が根として編成されるものと思わ

れる(第3図 c, e)、これはトマトの子葉での Carlson (1953) の観察と一致する。また佐藤(清)(1949) はスギの挿木で、カルスの中に根が形成されるときに、これと同じような過程で根ができることを見ている。生長点が形成されると根は生長してカルスを貫いて出る。根の原基がはじめて形成された時には、はじめはそれと葉脈との間に通導組織の連絡はない。しかしやがて両者の間に介在する細胞が仮導管に分化し、両者の連絡が作られるのであろう。側脈に出来たカルスからも同様にして次々に発根してくる。

不定根の中心柱の構造

子葉の葉脈端に生じた不定根は極めて細く貧弱なものが多い。そこでこれらを横断してその中心柱を見ると、その構造はいろいろの異常を示した。ササゲでは正常な根は、4 原型の中心柱をもっている。所が子葉から発生した不定根では、4 原型から 2 原型までの変化があつて、太い根は 4 原型、又はそれに近い構造、細いものは 3 原型又は 2 原型を示した(第 4 図)。これらの不定根から分れた側根も亦種々の型を示すが、太いものは 4 原型に近く、細いものはより簡単な型をもっていた。不定根及び中心柱の太さと、維管束数との相



第 4 図

関々係に関して、取扱つた根について得られた結果を次の表に示す。

Table 1. Diameter of roots and steles, and the patterns of the steles.

Diameter of root in μ	Diameter of stele in μ	Pattern of stele
Normal seedling roots		
930	430	tetrarch
900	300	tetrarch
740	320	tetrarch
Adventitious roots		
900	450	tetrarch
850	350	tetrarch
550	250	tetrarch
510	180	tetrarch
500	190	tetrarch
480	140	triarch
290	79	triarch
280	90	triarch
420	110	diarch
380	65	diarch
320	80	diarch

論 議

子葉は発芽植物において生長素の供給源である

ことが知られている。Nakano (1924) は子葉をいろいろな方向に切つてその切面から不定根の形成を研究し、カルスの形成には傷害ホルモンと共に、維管束を通じて供給されるホルモンが協同して働くこと、及びその量と質の差がより大きな効果をもつことを示した。Carlson (1953) の実験では、不定根のもと葉脈の切端部の篩部柔細胞群から作られることが示されている。これらのことにおいても根の形成は篩部とカルスとの間に葉脈の末梢にあることが示された。これらのことは、カルス及び不定根の形成が維管束の存在と極めて密接な関係をもつことを証明するものである。根の原基を形成すべき細胞又は細胞群に対してこの維管束を通じて、ホルモン及び營養物質が供給されるのであろう。もしこれらの物質の供給の多少によつて根の形成が影響されるとすれば、子葉の太い葉からは子葉の上葉片からよりも早くに発根するという La Rue (1923) の観察、及び太い主葉脈からは細い側脈からよりも早く発根するという Carlson (1943) 及び筆者の実験の結果は、それによつて説明することも出来るであろう。この物質供給量は単に子葉片の大小によるのではなくて、根の形成される部位に葉脈がどのように配置されてあるかによつて異なることであろう。

しかしそれと共に、不定根の原基となる篩部柔細胞群の量も、不定根の形成の容易さを決定する

う。分裂を起しうる柔細胞がより多量に存在する部位は、不定根の形成の容易さがより大いであるだろう。太い主葉脈の端からは細い側脈からよりも早く不定根が出てくることは、前者において篩部柔細胞の量がより多量にあることを示すものであるから上述の如くホルモンの供給と篩部柔細胞の量とが子葉からの発根の難易を左右するものであろう。

不定根の中心性の示す変異は、根の原基が、篩部柔細胞群から生じられるときに、通常の根の生長点と全く同じようには編成されないために起ると考えられる。一般に中心柱の原初木部の数は種々の植物によつて違つてゐるが、しかしそれは本質的なものではない (Esau, 1953)。維管束配列の型と根の太さ又は中心柱の太さとの相関関係が以前から論議されたが (Bower, 1930; Wardlaw, 1952), Thoday (1939) は維管束配列の型を作りだす分裂組織の直径こそが相関関係の分析に重要な量であつて、出来上つた根の中心柱の太さは問題ではないことを指摘した。また Torrey (1955) はエンドウの根の形成において、維管束型の決定は、型が作られるときの apical meristem の大きさに関係があることを暗示した。本研究においても、不定根の原基が十分に organize されるかどうかによつて、中心柱の異なる型が現れるのであろう。

Summary

1. Isolated cotyledons of *Vigna sinensis* were cultured on nutrient medium. When a part of the hypocotyl remained at the base of the cotyledon, the former grew out in a bulge, from which adventitious roots appeared.

2. When a cotyledon was cut at the base so as to remove entirely from the hypocotyl, the cut surface was covered by callus. In such a case, adventitious roots formed from the callus.

3. When a cotyledon was cut into apical and basal halves which are nearly equal in size, the cut surface of the apical pieces produced calluses and roots, while that of the basal pieces produced neither callus nor roots though they are formed at the opposite end.

4. Anatomical studies revealed that primordium of the adventitious root was differentiated from the parenchymatous cells of the phloem in the callus at the cut end of leaf vein.

5. It was discussed that the root formation might be influenced by the supply of hormone and other materials through the vein, and also by the amount of parenchymatous cells present in the phloem.

6. The number of arcs of the adventitious roots varied from four, a normal condition of the primary root, to two, intermediate conditions occurring also frequently.

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Explanations for figures

Fig. 1. a-f. Adventitious roots formed on the isolated cotyledons.

Fig. 2. Callus and root primordium formed on the isolated cotyledons with hypocotyl fragment.

a. Swelling of the hypocotyl fragment, cultured 3-days. $\times 13.4$

b. Initiation of lateral bud and root primordium, cultured 4 days.

b:bud; p: primordium of adventitious root. $\times 13.4$

c. Larger magnification of b. $\times 26$.

d. Root coming out of the swelling of the hypocotyl fragment, 5 days. $\times 13.4$

Fig. 3. Callus and root formation on the cut end of the leaf vein.

a. Cut end of the leaf vein, showing the tracheids filled with granules. Mesophyll cells contain starch grains. cultured 2 days. $\times 266$.

b. Cutinized cut surface and cell proliferation at the cut end of the vein. Starch grains have disappeared from the mesophyll cells in several layers under the cut surface, 4 days. $\times 266$.

c. Callus formed on the cut end of the vein, and the initiation of root primordium. p: root primordium. 5 days. $\times 66$.

d. Swelling of callus and protrusion of leaf vein at cut end of cotyledon, 4 days. $\times 13.4$

e. Callus and root primordium formed on the cut end of the vein. p: root primordium. 5 days. $\times 52$.

f. Root formed on the callus, 5 days. $\times 80$.

Fig. 4. Various patterns observed in the central cylinders of the roots.

a. Central cylinder of primary root of normal seedling, showing tetrarch vascular arrangement. $\times 80$.

b. Central cylinder of an adventitious root, showing tetrarch condition. $\times 160$.

c. Triarch central cylinder of an adventitious root. $\times 240$.

d, e. Diarch central cylinders of adventitious roots. $\times 160$.

Rapid Test of Free Asparagine in Plant Sap by a Fan Shape Paper Chromatography*

by Kiyoshi OZAKI**

尾 崎 清: 扇形ペーパークロマトグラフィーによる植物汁液中の
遊離アスパラギンの簡易検出法

Received November 12, 1955

The concept that asparagine and glutamine play different physiological roles in organisms is widely supported by many experimental results.¹⁾

The author has found in rice plant that glutamine has a principal role in the nitrogen metabolism whereas asparagine functions as a storage of excess nitrogen, appearing in the plant only when it takes up nitrogen in excess.²⁾ Asparagine was considered thus to be a good indicator to assess the nitrogen requirement of rice plant, this having been proved by the experiment.^{3,4)}

To apply this method in the field, a simple detecting method of asparagine was hoped for.

The author developed a paper chromatographic method for this purpose independently of the similar one which has been reported lately.⁵⁾

This method, which is mentioned below in detail, can also be applied for the detection of free asparagine in many other plant species.

Sampling: The plant leaves are cut and pinched very severely. The sap thus obtained is placed on a fan shape filter paper with a micro pipett or capillary tube. The sampling must be followed immediately by the pipetting, because sometimes asparagine has been detected in such a sample as showed normally no existence of asparagine, if they were laid away for four to six hours after the sampling. This may be caused by an abnormal decomposition of protein. The procedure, therefore, must be finished within an hour in any case.

Filter paper: The filter paper used in the present study was No. 5A (15 cm in diameter) made by Toyo Roshi Co, Ltd. (a rapid filtering paper of loose texture.)

As shown in the Fig. 1 (a) (b), the filter paper was cut into six pieces of fan shape.

Solvent: The n-butanol-acetic-water solvent⁶⁾ is modified as follows: n-butanol including 0.4% ninhydrine, glacial acetic acid and water were mixed 4:1:1 in volume

* This research was reported at the meeting of the Society of the Science of Soil and Manure on April, 1955.

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ratios. To save the spray after development ninhydrine is added into the solvent beforehand.

Development: As shown in the Fig. 1 (c), dip the narrow part of the filter paper into the solvent and let to stand until the solvent moves almost to the arc end.

Usually the development requires about thirty minutes 27-30°C.

Colour development and identification: After the development the paper is heated to identify amides and amino acids spots.

It is quite easy to find out the asparagine spot, if it exists, because R_f of asparagine in n-butanol-acetic-water solvent is the least among such amino acids and amides as are usually contained in plant sap, and because the asparagine spot exhibits greyish blue colour distinct from purplish blue spots of others.

If the more precise detection is required, chemical pure asparagine should be run on the same paper as the standard.

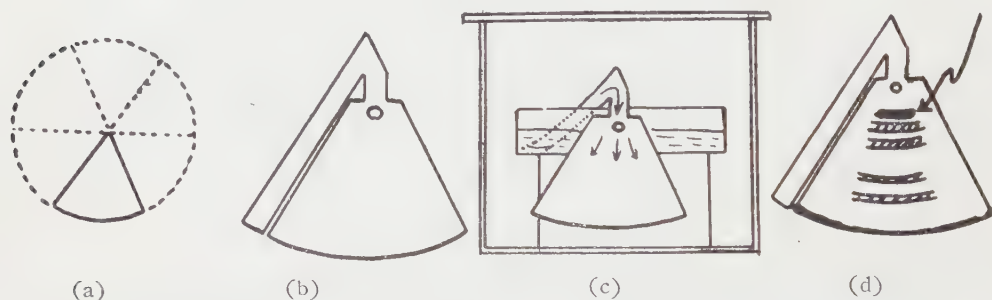


Fig. 1. (a) No. 5A filter paper (15 cm in diameter) is cut into six pieces. (b) Each piece is cut out like the figure. The sample is placed on the spot. (c) Development. The narrow part of the filter paper is dipped into the solvent held in petri dish. (d) Identification of asparagine.

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Perforating Growth of *Conchocelis* in Calcareous Matrices

by Eizi OGATA*

尾形英二：石灰質中におけるコンコセリスの穿孔成長

Received December 12, 1955.

It was stated by Drew (1953) that the *Conchocelis*-phase of a species of *Porphyra* was found in the peduncular scales of the barnacle, *Pollicipes cornucopia*, and that this *Conchocelis*-phase showed a close resemblance to *C. rosea* Batters, an alga frequently perforating in variety of matrices such as shells, calcareous algae, calcareous stone, etc. In our laboratory, perforating growth of filamentous germlings from spores of *Porphyra* and *Bangia* was investigated experimentally, using thin plates of calcite and lime stone. Material plants were obtained from Osaka Bay. For preparation of matrix substratum, pieces of transparent calcite and lime stone which had previously been cemented on glass slides were ground as thin as 200 to 300 μ by means of a grinder. These matrices facilitated microscopic observations on growing filaments by transmitted light.

With these material plants and matrices subsequent experiments were carried out since March 1955. The results obtained are as follows; 1. Spores liberated from *Porphyra tenera*, collected on March 24, gave rise to be so-called germ tubes, which immediately penetrated into the lime stone plate and grew up to *Conchocelis* filaments. The growth patterns were almost similar to the case in oyster shell.

2. Filaments occurred from the spores of *Porphyra tenera* which had been obtained on April 5 also penetrated immediately into the inner layer of calcite and grew up to ordinary *Conchocelis* form as the case in the inner layer of oyster shells in natural habitat. Spores germinated on slides at the same time developed creeping filaments in a way similar to the previous description by Kurogi (1953).

Differences in form and behaviour of growth were little between natural and experimental ones, although the latter seemed more obtuse in the whole shape.

It is of interest that the direction of branch growth seemed to have some connection with the cleavage plane of calcite. That is, some of the marginal filaments, which had been growing at right angle towards the direction of cleavage plane or the interstice-like lines, turned the direction and grew along them, and vice versa.

3. *Conchocelis* filaments in these calcite and lime stone matrices survived until this autumn through the summer. However, dead filaments, faded in colour, were found abundantly in calcite more than in shell matrix.

4. The first experiment on *Bangia fusco-purpurea* was performed with the material collected on April 5. The germination of spores of this species gave rise

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to filaments which penetrated into calcite matrix, showing considerably more rapid and extensive growth than in *Porphyra*. In such a matrix they developed into extensive colonies, which were more reddish in colour and obtuser and broader than *Conchocelis* filaments of *Porphyra*.

It is of interest that the bell-like inflations arose on the secondary branches of the filaments in the early stage of development.

In the case of this species the direction of cleavage plane of calcite appeared to have more remarkable relation to the direction of growth in branching than in the case of the filament of *Porphyra* in calcite. Particularly, interstices due to cleavage plane seemed to have a close relation with the direction of branch growth. Unlike the radial development in oyster shell, more or less square colonies of filaments developed in the calcite matrix. But such a tendency is not absolutely constant but seems rather fortuitous.

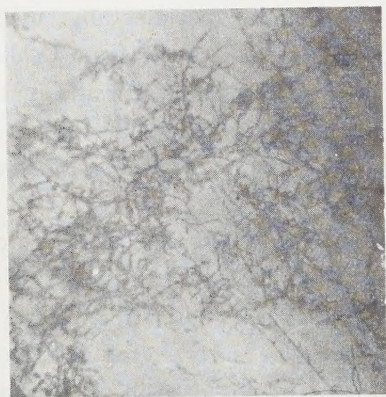


Fig. 1. *Conchocelis* filaments of *Bangia fusco-purpurea* in calcite matrix showing the branch growth influenced by cleavage direction. $\times 60$ (Photographed on May 26).

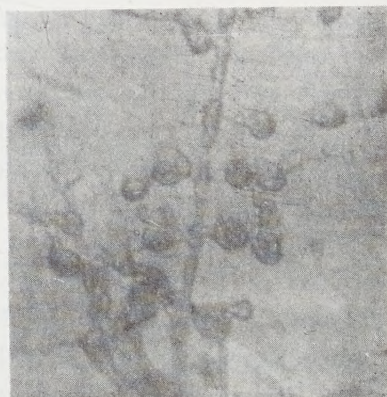


Fig. 2. Inflated branches of *Conchocelis* filaments of *Bangia fusco-purpurea* in calcite matrix. $\times 250$. (Photographed on May 26).

These filaments survived through this summer and "fertile cell-rows" (termed by Drew) occurred on these filaments in this autumn although the dead filaments, faded in colour, were more abundant than in shell matrix. On November 7, numerous uniseriate upright plantlets became observable on the calcite surface. These doubtlessly occurred by germination of "monospores" (termed by Kurogi) from fertile cell-rows of *Conchocelis* filaments of *Bangia* employed.

As considered from the above results, germlings from both *Porphyra* and *Bangia* are usually able to penetrate into such inorganic calcareous matrices as calcite and lime stone, and to grow as normally as in the shell matrix. Further investigation upon the mechanism of penetration into substratum matrix is now in progress.

I wish to express here my hearty thanks to Dr. S. Segawa for his kind advice and to Prof. S. Miki and Assoc. Prof. S. Nagai for their continuous encouragements.

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